PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, C07K 14/715, A61K 48/00, C07K 16/28, G01N 33/50, A61K 39/395

A2

(11) International Publication Number: WO 99/06561

(43) International Publication Date: 11 February 1999 (11.02.99)

(21) International Application Number: PCT/US98/15730

(22) International Filing Date: 29 July 1998 (29.07.98)

(30) Priority Data:
60/054,094 29 July 1997 (29.07.97) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 60/054,094 (CON)
Filed on 29 July 1997 (29.07.97)

(71) Applicant *(for all designated States except US)*: THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES, NATIONAL INSTITUTES OF HEALTH Office of Technology Transfer [US/US]; Suite 325, 6011 Executive Boulevard, Rockville, MD 20852–3804 (US).

(72) Inventors; and

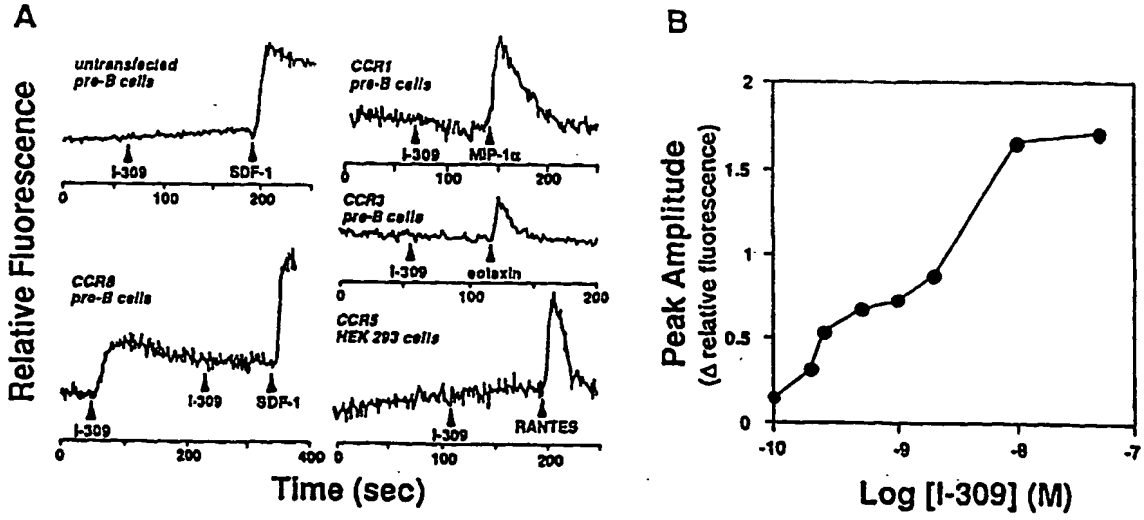
(75) Inventors/Applicants *(for US only)*: TIFFANY, H., Lee [US/US]; 13910 Congress Drive, Rockville, MD 20853–2633 (US). MURPHY, Philip, M. [US/US]; 10 Hastings Circle, Rockville, MD 20850 (US). BERGER, Edward, A. [US/US]; 5820 Inman Park Circle, Rockville, MD 20852 (US). ALKHATIB, Ghalib [CA/US]; 13264 Penneagle Drive, Carmel, IN 46033 (US). BAZAN, Hernan [US/US]; Apartment 101, 3338 O Street, N.W., Washington, DC 20007 (US). BONNER, Tom, I. [US/US]; Bethesda, MD (US). LAUTENS, Laura [US/US]; The Plains, OH (US).

(74) Agent: HAILE, Lisa, A.; Fish & Richardson P.C., Suite 1400, 4225 Executive Square, La Jolla, CA 92037 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: CHEMOKINE RECEPTOR CCR8 DNA AND USES THEREOF



(57) Abstract

The susceptibility of target to human immunodeficiency virus (HIV) infection depends on cell surface expression of the human CD4 molecule and CC chemokine receptor 8. CCR8 is a member of the 7–transmembrane segment superfamily of G–protein–coupled cell surface molecules. CCR8 plays an essential role in the membrane fusion step of infection by diverse HIV isolates. The establishment of stable, nonhuman cell lines and transgenic mammals having cells that coexpress human CD4 and CCR8 provides valuable tools for the continuing research of HIV infection. In addition, antibodies which bind to CCR8, CCR8 variants, and CCR8–binding agents, capable of blocking membrane fusion between HIV and target cells represent potential anti–HIV therapeutics.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

CHEMOKINE RECEPTOR CCR8 DNA AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates to *in vitro* and *in vivo* models for the study of human immunodeficiency virus (HIV) infection and the effectiveness of anti-HIV therapeutics. The invention more specifically relates to cell surface proteins that participate in HIV infection and which are useful for the development of animal models.

BACKGROUND OF THE INVENTION

An HIV infection cycle begins with the entry of an HIV virus into a target cell. Entry commences when an HIV envelope glycoprotein (*env*) binds to a human CD4 molecule in a target cell membrane. This binding leads to fusion of virus and cell membranes, which in turn facilitates virus entry into the host. The HIV-infected host cell eventually expresses *env* on its surface. This expression allows the infected cell to fuse with uninfected, CD4-positive cells, thereby spreading the virus.

Recent studies have shown that the HIV fusion process occurs with a wide range of human cell types that either express human CD4 endogenously or that have been engineered to express human CD4. The fusion process, however, does not occur with nonhuman cell types engineered to express human CD4 even though these nonhuman cells still can bind *env*. The disparity between human and nonhuman cell types exists because membrane fusion requires the coexpression of human CD4 and one or more cofactors specific to human cell types. Nonhuman cell types that have been engineered to express human CD4 but not the additionally required factor(s) are incapable of membrane fusion, and therefore are nonpermissive for HIV infection.

Some individual HIV isolates, designated "macrophage-tropic," efficiently infect primary macrophages but not immortalized T-cell lines. Other isolates, designated "T-cell line-tropic," have the opposite property and infect immortalized T-cell lines more efficiently than they infect primary macrophages. Both types of isolates readily infect primary T-cells from the body, however. The selective tropism of these two types of isolates is thought to be due to their requirements for distinct cofactors that are differentially expressed on different CD4 positive cell types. It should be understood that other HIV strains are "dual-tropic" and have the ability to infect both macrophages and immortalized T-cell lines and are believed to be able to use more than one cofactor.

In recent years, researchers have bred transgenic animals that contain cells which express human CD4 and which could be used as models for HIV infection of macrophages if the macrophage-specific factor were known. See, for example, Dunn *et al.*, *Human immunodeficiency virus type 1 infection of human CD4-transgenic rabbits*, J. Gen. Vir. 76:1327-1336 (1995); Snyder *et al.*, *Development and Tissue-Specific Expression of Human CD4 in Transgenic Rabbits*, Mol. Reprod. & Devel. 40:419-428 (1995); Killeen *et al.*, *Regulated Expression of Human CD4 Rescues Helper T-Cell Development in Mice Lacking Expression of Endogenous CD4*, EMBO J. 12:1547-1553 (1993); Forte *et al.*, *Human CD4 Produced in Lymphoid Cells of Transgenic Mice Binds HIV gp120 and Modifies the Subsets of Mouse T-Cell Populations*, Immunogenetics 38:455-459 (1993).

A goal of research in this field is to find a putative factor for HIV isolates that could be co-expressed with CD4 in a small animal. Such co-expression would provide an animal model to develop efficacious therapies to combat infection by macrophage-tropic and/or T-cell tropic HIV isolates. The discovery of other essential cofactors would provide new targets for development of anti-HIV therapies.

The chemokine superfamily consists of specific leukocyte chemoattractant proteins that can be sorted by structure into four groups, designated C, CX_3C, CC and CXC depending on the number and spacing of conserved cysteines (Baggiolini *et al.*, *Interleukin-8 and*

related chemotactic cytokines--CXC and CC chemokines, Adv. Immunol. 55:97-179 (1994); Bazan *et al.*, *A new class of membrane-bound chemokine with a CXC3C motif*, Nature 385:640-644 (1997); Kelner *et al.*, *Lymphotactin: a cytokine that represents a new class of chemokine*, Science 266:1395-1398 (1994)). The C and CX_3C groups each have only one known member, whereas the CC and CXC groups each have many members. CXC chemokines mainly target neutrophils and T cells, and C and CX_3C chemokines are specific for T cells. CC chemokines target monocytes, eosinophils, basophils and T cells with variable selectivity, but, in most cases, they do not target neutrophils.

I-309 is a human CC chemokine first identified by molecular cloning in a search for genes expressed in activated T cell lines (Miller *et al.*, *Sequence and chromosomal location of the I-309 gene. Relationship to genes encoding a family of inflammatory cytokines*, J. Immunol. 145:2737-2744 (1990)). Like other CC chemokines, I-309 induces chemotaxis in monocytes (Miller *et al.*, *The human cytokine I-309 is a monocyte chemoattractant*, Proc. Natl. Acad. Sci. USA 89:2950-2954 (1992)). Recently, I-309 was purified from CD4+ T cells as a secreted factor that protects murine thymic lymphoma cell lines from dexamethasone-induced apoptosis (Van Snick *et al.*, *I-309/T cell activation gene-3 chemokine protects murine T cell lymphomas against dexamethasone-induced apoptosis*, J. Immunol. 157:2570-2576 (1996)). Other chemokines have little or no activity, suggesting a unique signaling pathway.

The first step in chemokine action involves binding to G protein-coupled receptors on the cell surface. Five CC chemokine receptors have been reported, CCR1-5, all of which are expressed on leukocytes (Neote *et al.*, *Molecular cloning, functional expression, and signaling characteristics of a C-C chemokine receptor*, Cell 72:415-425 (1993); Gao *et al.*, *Structure and functional expression of the human macrophage inflammatory protein-1α/RANTES receptor*, J. Exp. Med. 177:1421-1427 (1993); Charo *et al.*, *Molecular cloning and functional expression of two monocyte chemoattractant protein 1 receptors reveals alternative splicing of the carboxyl-terminal tails*, Proc. Natl. Acad. Sci. USA

91:2752-2756 (1994); Kitaura *et al.*, *Molecular cloning of human eotaxin, an eosinophil-selective CC chemokine, and identification of a specific eosinophil eotaxin receptor, CC Chemokine Receptor 3*, J. Biol. Chem. 271:7725-7730 (1996); Ponath *et al.*, *Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils*, J. Exp. Med. 183:2437-2448 (1996); Power *et al.*, *Molecular cloning and functional expression of a novel CC chemokine receptor cDNA from a human basophilic cell line*, J. Biol. Chem. 270:19495-19500 (1995); Samson *et al.*, *Molecular cloning and functional expression of a new human CC-chemokine receptor gene*, Biochemistry 35:3362-3367 (1996); Combadiere *et al.*, *Cloning and functional expression of CC CKR5, a human monocyte CC chemokine receptor selective for MIP-1α, MIP-1β, and RANTES*, J. Leukocyte Biol. 60:147-152 (1996)). Each receptor has multiple ligands, and the ligand repertoires overlap extensively among receptors. Nevertheless, none of these receptors has been shown to interact with I-309.

SUMMARY OF THE INVENTION

The present invention is based on the discovery of a new CC chemokine receptor protein associated with HIV infection, known as "CCR8". The human CC chemokine I-309 is a potent monocyte chemoattractant and inhibits apoptosis in thymic cell lines. The present invention provides a human I-309 receptor, CCR8, named according to an accepted nomenclature system in the art. The receptor has 7 predicted transmembrane domains, is expressed constitutively in monocytes and thymus, and is encoded by a gene of previously unknown function, named alternatively CY6, TER1 and CKR-L1 (Napolitano *et al.*, *Molecular cloning of TER1, a chemokine receptor-like gene express by lymphoid tissues*, J. Immunol. 157:2759-2763 (1996); Zaballos *et al.*, *Molecular cloning and RNA expression of two new human chemokine receptor-like genes*, Biochem. Biophys. Res. Commun. 227:846-853 (1996)). After transfection with the CY6 open reading frame, a mouse pre-B cell line exhibited calcium flux and chemotaxis in response to I-309 (EC_{50}=2 nM for each), whereas 20 other chemokines were inactive. Signaling was sensitive to pertussis toxin, suggesting coupling to a G_i-type G protein. These

properties parallel those of endogenous I-309 receptors expressed in an HL-60 clone 15 cell line model. The apparent monogamous relationship between I-309 and CCR8 is unique among known CC chemokines and known CC chemokine receptors. CCR8 may regulate monocyte chemotaxis and thymic cell line apoptosis.

The invention provides isolated polynucleotides and polypeptides encoded by CCR8 polynucleotides, as well as antibodies directed against regions of CCR8 and peptide fragments of CCR8 which block HIV interaction with the CCR8 receptor.

It is an object of the present invention to provide therapeutic and preventative medicinal agents effective against HIV infection and effective in regulating monocyte accumulation and activation. In accomplishing these and other objects, there has been provided, in accordance with one aspect of the present invention a stable, nonhuman cell line; the cells of which contain DNA encoding CCR8. In accordance with another aspect of the invention a transgenic non-human mammal is provided comprised of cells that coexpress human CD4 and CCR8.

In another aspect of the invention, the invention provides an antibody which binds to CCR8 and which blocks membrane fusion between HIV and a target cell. In accordance with another aspect of the invention, there is provided a cell that expresses a CCR8 gene, wherein the CCR8 gene is not stably integrated into the genome of said cell.

In accordance with yet another aspect of the invention isolated and purified peptide fragments of CCR8 are provided that block membrane fusion between HIV and a target cell.

In yet another aspect, the invention provides a method for identifying a compound which blocks membrane fusion between HIV and a CCR8 target cell or between an HIV-infected cell and a CCR8 positive uninfected cell. The method includes the steps of : a) incubating components comprising the compound and a CD4 and CCR8 positive cell

under conditions sufficient to allow the components to interact; b) contacting the components of step a) with HIV or an HIV envelope-expressing cell; and c) measuring the ability of the compound to block membrane fusion between HIV and the CCR8 positive cell or between an HIV envelope expressing cell and a CCR8 positive uninfected cell.

In accordance with yet another aspect of the invention a method of inhibiting CCR8 expression in a cell is provided, comprising introducing into the cell at least one antisense polynucleotide that causes the inhibition of CCR8 in the cell.

In accordance with yet another aspect of the invention is provided a CCR8-binding agent, wherein said agent blocks binding of a chemokine and HIV to CCR8. It also is an objective of the present invention to isolate and purify CCR8-binding agents, both biologic and chemical compounds, that block membrane fusion between HIV and a target cell or beween an HIV infected cell and an uninfected CD4 positive cell. A biologic agent of the invention includes I-309, which is a natural ligand for CCR8.

The antibodies and blocking agents of the invention are also useful for providing methods for modulating an immune response. For example, administration of CCR8 agonists or antagonists would be useful for modulating the immune response.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. RNA distribution of CCR8. Northern blots containing total RNA from the sources indicated above each lane were hybridized with a CCR8 ORF probe under high stringency conditions. M, monocyte/macrophages (PBMCs that remained adherent to plastic after 18 h overnight culture); L, lymphocytes (non-adherent PBMCs); N, neutrophils. The blots were exposed for 3 d to X-ray film using an intensifying screen.

Figure 2. I-309 is an agonist for CCR8. *A*) Receptor specificity and homologous densensitization. $[Ca^{2+}]_i$ was monitored by ratio fluorescence of FURA-2 loaded pre-B cells or HEK 293 cells stably transfected with plasmids encoding CC chemokine receptors as indicated adjacent to each tracing. Cells were stimulated with chemokines 50 nM at the times indicated by arrowheads. Data are representative of at least three experiments with CCR8-expressing cells. *B*) Potency. The amplitude of the peak of the calcium transient elicited by the indicated concentration of I-309 in CCR8 transfectants is shown. Data are representative of 2 separate experiments.

Figure 3. CCR8 is a chemotactic receptor. Untransfected pre-B cells (open squares) and cells stably expressing CCR8 (closed circles) were incubated in a microchemotaxis chamber and tested with the indicated concentrations of I-309. The number of input cells was 350,000/well. Data are the mean +/- SEM of triplicate determinations, and are from a single experiment representative of two separate experiments. Checkerboard analysis indicated that the activity was chemotactic, not chemokinetic (not shown).

Figure 4. An HL-60 clone 15 cell line model of endogenous CCR8 expression and function. HL-60 clone 15 cells were cultured for 6 d in the presence of butyric acid 0.5 μM and for the final 4 d in the presence of IL-5 10 ng/ml, which induces differentiation to an eosinophilic phenotype. *A*, CCR8 mRNA expression. A Northern blot containing 10 μg total RNA from undifferentiated (U) and differentiated (D) cells was hybridized with a CCR8 ORF probe (top) and washed under high stringency conditions. The blot

was then exposed to X-ray film using an intensifying screen for 20 h. The corresponding region of the ethidium bromide-stained gel is shown in the lower panel. *B*) Calcium flux response to I-309. Fura-2-loaded undifferentiated (top tracing, U) and differentiated (lower tracing, D) cells were stimulated with I-309 50 nM. *C*) Potency of I-309 for calcium flux. Data are from a single experiment representative of 3 separate experiments. *D*) Distinct receptor usage by I-309 and other CC chemokines. Differentiated cells were stimulated with the indicated chemokines 50 nM and Fura-2 fluoresence was monitored.

Figure 5. CCR8 Couples to a G_i-type G protein. $[Ca^{2+}]_i$ was measured as the relative fluorescence emitted by Fura-2-loaded pre-B cells stably transfected with CCR8 or HL-60 clone 15 cells differentiated with butyric acid and IL-5 after 4 h treatment with the inhibitors indicated to the right of each tracing. I-309 50 nM was added at the time indicated by the arrow. The results are from a single experiment representative of 3 separate experiments.

Figure 6. HIV-1 coreceptor activity of CCR8: cell-cell fusion activity. Effector NIH 3T3 cells expressing the HIV-1 envelope glycoproteins indicated on the x-axis were mixed with target NIH 3T3 cells expressing CD4 and CCR8, and cell-cell fusion was measured. UNC, an uncleavable mutant envelope protein from strain IIIB.

Figure 7. HIV-1 coreceptor activity of CCR8: activity compared to other HIV-1 coreceptors. Effector NIH 3T3 cells expressing the HIV-1 envelope glycoproteins indicated on the x-axis were mixed with target NIH 3T3 cells expressing CD4 and the chemokine receptor given by the code at the upper right, and cell-cell fusion was measured. UNC, an uncleavable mutant envelope protein from strain IIIB.

Figure 8. Inhibition of CCR8's HIV-1 coreceptor activity by the CCR8 ligand I-309. Effector NIH 3T3 cells expressing the HIV-1 envelope glycoproteins indicated on the x-axis were mixed with target NIH 3T3 cells expressing CD4 and CCR8 in the presence

and absence of the chemokines indicated by the code to the right, and cell-cell fusion was measured.

Figure 9. Dose-dependent inhibition of CCR8's HIV-1 coreceptor activity by the CCR8 ligand, I-309.

Figure 10A and 10B. Nucleotide (10A) and deduced amino acid (10B) sequence of CCR8.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention originated from studies on receptor proteins of chemokines. The inventors cloned, sequenced, and functionally expressed a human cDNA encoding a novel CC chemokine receptor that has been designated CCR8.

During their investigation, the inventors discovered that CCR8 is a necessary cofactor for infection by HIV isolates. More particularly, the inventors found that when they transgenically expressed human CCR8 in non-human cells which also transgenically express human CD4, the altered cells could fuse with cells that express the *env* envelope protein from diverse strains of HIV. It should be understood that HIV strains can be classified as "dual-tropic" and have the ability to infect both macrophages and immortalized T-cell lines, macrophage-tropic, or T-cell tropic, and are believed to be able to use more than one cofactor. CCR8 can interact with envelopes from all three classes of HIV-1. Furthermore, the inventors reasoned that antibodies against CCR8 can inhibit the fusion of cells that contain CD4 and CCR8, upon contact with cells that express the *env* protein from macrophage-tropic strains of HIV. Antibodies which bind CCR8 can inhibit infection of cells that contain CCR8 and CD4 by HIV. The insights of the present invention enable the development of new tools to study HIV infection and the discovery of new HIV treatment methodologies based on chemokine receptor biochemistry.

Chemokine receptors are thought to have seven transmembrane-domains, are coupled to G-protein and participate in cellular responses to chemokines.

ISOLATION OF cDNA ENCODING CCR8

A nucleotide sequence determined by the inventors, herein described as SEQ ID NO:1 of the present invention, has been deposited with the Genbank/EMBL data libraries under accession number U45983 (Figure 10). But many other related sequences that code for CCR8 and altered forms of CCR8 are contemplated in context of the various embodiments enumerated herein.

In preferred embodiments fusion between *env*-expressing effector cells and CD4-expressing and CCR8-expressing target cells, prepared by infection with vaccinia virus, induces activation of *Escherichia coli* lacZ, causing β-galactosidase production in fused cells as described by Nussbaum *et al.*, *J. Virol.* 68: 5411 (1994), which is incorporated in its entirety by reference. The specificity of cell fusion as measured with this assay is equivalent to the specificity of infection by HIV-1 virions.

The invention provides an isolated polynucleotide sequence encoding a polypeptide having an amino acid sequence as set forth in SEQ ID NO:2. The term "isolated" as used herein includes polynucleotides substantially free of other nucleic acids, proteins, lipids, carbohydrates or other materials with which it is naturally associated. Polynucleotide sequences of the invention include DNA, cDNA and RNA sequences which encode CCR8. It is understood that all polynucleotides encoding all or a portion of CCR8 are also included herein, as long as they encode a polypeptide with CCR8 activity (*e.g.*, act as a cofactor for HIV infection). Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated polynucleotides. For example, portions of the mRNA sequence may be altered due to alternate RNA splicing patterns or the use of alternate promoters for RNA transcription. As another example, CCR8 polynucleotide may be subjected to site-directed mutagenesis. The polynucleotide sequence for CCR8

also includes antisense sequences. The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of CCR8 polypeptide encoded by the nucleotide sequence is functionally unchanged. Also included are nucleotide sequences which encode CCR8 polypeptide, such as SEQ ID NO:1. In addition, the invention also includes a polynucleotide encoding a polypeptide having the biological activity of an amino acid sequence of SEQ ID NO:3 and having at least one epitope for an antibody immunoreactive with CCR8 polypeptide. Assays provided herein which show association between HIV infection and expression of CCR8 can be used to detect CCR8 activity.

The polynucleotide encoding CCR8 includes the nucleotide sequence in FIGURE 10 (SEQ ID NO:1), as well as nucleic acid sequences complementary to that sequence. A complementary sequence may include an antisense nucleotide. When the sequence is RNA, the deoxyribonucleotides A, G, C, and T of FIGURE 10 are replaced by ribonucleotides A, G, C, and U, respectively. Also included in the invention are fragments (portions) of the above-described nucleic acid sequences that are at least 15 bases in length, which is sufficient to permit the fragment to selectively hybridize to DNA that encodes the protein of FIGURE 10 (*e.g.*, SEQ ID NO:2). "Selective hybridization" as used herein refers to hybridization under moderately stringent or highly stringent physiological conditions (See, for example, the techniques described in Maniatis et al., 1989 Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y., incorporated herein by reference), which distinguishes related from unrelated nucleotide sequences.

In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity, nucleotide sequence composition (*e.g.*, GC v. AT content), and nucleic acid type (*e.g.*, RNA v. DNA) of the hybridizing

regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.

An example of progressively higher stringency conditions is as follows: 2 x SSC/0.1% SDS at about room temperature (hybridization conditions); 0.2 x SSC/0.1% SDS at about room temperature (low stringency conditions); 0.2 x SSC/0.1% SDS at about 42°C (moderate stringency conditions); and 0.1 x SSC at about 68°C (high stringency conditions). Washing can be carried out using only one of these conditions, *e.g.*, high stringency conditions, or each of the conditions can be used, *e.g.*, for 10-15 minutes each, in the order listed above, repeating any or all of the steps listed. However, as mentioned above, optimal conditions will vary, depending on the particular hybridization reaction involved, and can be determined empirically.

DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated using hybridization or computer-based techniques which are well known in the art. These include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences; 2) antibody screening of expression libraries to detect cloned DNA fragments with shared structural features; 3) polymerase chain reaction (PCR) on genomic DNA or cDNA using primers capable of annealing to the DNA sequence of interest; 4) computer searches of sequence databases for similar sequences; and 5) differential screening of a subtracted DNA library.

Preferably the CCR8 polynucleotide of the invention is derived from a mammalian organism. Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Oligonucleotide probes, which correspond to a part of the sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of amino acid sequence must be known. The DNA sequence

encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, *et al., Nucl. Acid Res.*, 9:879, 1981). Alternatively, a subtractive library, as illustrated herein is useful for elimination of non-specific cDNA clones.

When the entire sequence of amino acid residues of the desired polypeptide is not known, the direct synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay, *et al., Nucl. Acid Res.*, 11:2325, 1983).

A cDNA expression library, such as lambda gt11, can be screened indirectly for CCR8 peptides having at least one epitope, using antibodies specific for CCR8. Such

antibodies can be either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of CCR8 cDNA.

Alterations in CCR8 nucleic acid include intragenic mutations (*e.g.*, point mutation, nonsense (stop), missense, splice site and frameshift) and heterozygous or homozygous deletions. Detection of such alterations can be done by standard methods known to those of skill in the art including sequence analysis, Southern blot analysis, PCR based analyses (*e.g.*, multiplex PCR, sequence tagged sites (STSs)) and *in situ* hybridization. Such proteins can be analyzed by standard SDS-PAGE and/or immunoprecipitation analysis and/or Western blot analysis, for example.

DNA sequences encoding CCR8 can be expressed *in vitro* by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

In the present invention, the CCR8 polynucleotide sequences may be inserted into a recombinant expression vector. The term "recombinant expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the CCR8 genetic sequences. Such expression vectors contain a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg, *et al.*, *Gene* ,56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.*, 263:3521, 1988) and baculovirus-derived vectors for

expression in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter (*e.g.*, T7, metallothionein I, or polyhedrin promoters).

Polynucleotide sequences encoding CCR8 can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. However, since mature CCR8 is glycosylated, the choice of host cells depends on whether or not the glycosylated or non-glycosylated form of CCR8 is desired. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention.

Methods which are well known to those skilled in the art can be used to construct expression vectors containing the CCR8 coding sequence and appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* recombination/genetic techniques. (See, for example, the techniques described in Maniatis et al., 1989 Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y.)

A variety of host-expression vector systems may be utilized to express the CCR8 coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing the CCR8 coding sequence; yeast transformed with recombinant yeast expression vectors containing the CCR8 coding sequence; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing the CCR8 coding sequence; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the CCR8 coding sequence; or animal cell systems infected with recombinant virus

expression vectors (*e.g.*, retroviruses, adenovirus, vaccinia virus) containing the CCR8 coding sequence, or transformed animal cell systems engineered for stable expression. Since CCR8 has not been confirmed to contain carbohydrates,both bacterial expression systems as well as those that provide for translational and post-translational modifications may be used; *e.g.*, mammalian, insect, yeast or plant expression systems.

Depending on the host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, etc. may be used in the expression vector (see *e.g.*, Bitter et al., 1987, Methods in Enzymology 153:516-544). For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage γ, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used. When cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the inserted CCR8 coding sequence.

In yeast, a number of vectors containing constitutive or inducible promoters may be used. For a review see, Current Protocols in Molecular Biology, Vol. 2, 1988, Ed. Ausubel et al., Greene Publish. Assoc. & Wiley Interscience, Ch. 13; Grant et al., 1987, Expression and Secretion Vectors for Yeast, in Methods in Enzymology, Eds. Wu & Grossman, 31987, Acad. Press, N.Y., Vol. 153, pp.516-544; Glover, 1986, DNA Cloning, Vol. II, IRL Press, Wash., D.C., Ch. 3; and Bitter, 1987, Heterologous Gene Expression in Yeast, Methods in Enzymology, Eds. Berger & Kimmel, Acad. Press, N.Y., Vol. 152, pp. 673-684; and The Molecular Biology of the Yeast Saccharomyces, 1982, Eds. Strathern et al., Cold Spring Harbor Press, Vols. I and II. A constitutive yeast promoter such as ADH or LEU2 or an inducible promoter such as GAL may be used (Cloning in Yeast, Ch. 3, R. Rothstein In: DNA Cloning Vol.11, A Practical Approach, Ed. DM Glover, 1986, IRL

Press, Wash., D.C.). Alternatively, vectors may be used which promote integration of foreign DNA sequences into the yeast chromosome.

Eukaryotic systems, and preferably mammalian expression systems, allow for proper post-translational modifications of expressed mammalian proteins to occur. Eukaryotic cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, phosphorylation, and advantageously, plasma membrane insertion of the gene product may be used as host cells for the expression of CCR8.

Mammalian cell systems which utilize recombinant viruses or viral elements to direct expression may be engineered. For example, when using adenovirus expression vectors, the CCR8 coding sequence may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. Alternatively, the vaccinia virus 7.5K promoter may be used. (*e.g.*, see, Mackett et al., 1982, Proc. Natl. Acad. Sci. USA 79: 7415-7419; Mackett et al., 1984, J. Virol. 49: 857-864; Panicali et al., 1982, Proc. Natl. Acad. Sci. USA 79: 4927-4931). Of particular interest are vectors based on bovine papilloma virus which have the ability to replicate as extrachromosomal elements (Sarver, et al., 1981, Mol. Cell. Biol. 1: 486). Shortly after entry of this DNA into mouse cells, the plasmid replicates to about 100 to 200 copies per cell. Transcription of the inserted cDNA does not require integration of the plasmid into the host's chromosome, thereby yielding a high level of expression. These vectors can be used for stable expression by including a selectable marker in the plasmid, such as, for example, the neo gene. Alternatively, the retroviral genome can be modified for use as a vector capable of introducing and directing the expression of the CCR8 gene in host cells (Cone & Mulligan, 1984, *Proc. Natl. Acad. Sci. USA* 81:6349-6353). High level expression may also be achieved using inducible promoters, including, but not limited to, the metallothionine IIA promoter and heat shock promoters.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. Rather than using expression vectors which contain viral origins of

replication, host cells can be transformed with the CCR8 cDNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. For example, following the introduction of foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler,*et al.*, 1977, *Cell* 11: 223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, *Proc. Natl. Acad. Sci. USA* 48: 2026), and adenine phosphoribosyltransferase (Lowy, *et al.*, 1980, *Cell* 22: 817) genes can be employed in tk-, hgprt$^-$ or aprt$^-$ cells respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, *et al.*, 1980, *Natl. Acad. Sci. USA* 77: 3567; O'Hare, *et al.*, 1981, *Proc. Natl. Acad. Sci. USA* 78: 1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78: 2072; neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, *et al.*, 1981, *J. Mol. Biol.* 150: 1); and hygro, which confers resistance to hygromycin (Santerre, *et al.*, 1984, *Gene* 30: 147) genes. Recently, additional selectable genes have been described, namely trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, 1988, *Proc. Natl. Acad. Sci. USA* 85: 8047); and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue L., 1987, In: Current Communications in Molecular Biology, Cold Spring Harbor Laboratory ed.).

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding the CCR8 of

the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein. (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

Cell Lines

In one embodiment, the present invention relates to stable recombinant cell lines, the cells of which express CCR8 polypeptide or coexpress human CD4 and CCR8 and contain DNA that encodes CCR8. Suitable cell types include but are not limited to cells of the following types: NIH 3T3 (Murine), Mv 1 lu (Mink), BS-C-1 (African Green Monkey) and human embryonic kidney (HEK) 293 cells. Such cells are described, for example, in the Cell Line Catalog of the American Type Culture Collection (ATCC). These cells can be stably transformed by a method known to the skilled artisan. See, for example, Ausubel *et al.*, *Introduction of DNA Into Mammalian Cells*, in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, sections 9.5.1-9.5.6 (John Wiley & Sons, Inc. 1995). "Stable" transformation in the context of the invention means that the cells are immortal to the extent of having gone through at least 50 divisions.

CCR8 can be expressed using inducible or constituitive regulatory elements for such expression. Commonly used constituitive or inducible promoters, for example, are known in the art. The desired protein encoding sequence and an operably linked promoter may be introduced into a recipient cell either as a non-replicating DNA (or RNA) molecule, which may either be a linear molecule or, more preferably, a closed covalent circular molecule. Since such molecules are incapable of autonomous replication, the expression of the desired molecule may occur through the transient expression of the introduced sequence. Alternatively, permanent expression may occur through the integration of the introduced sequence into the host chromosome. Therefore the cells can be transformed stably or transiently.

An example of a vector that may be employed is one which is capable of integrating the desired gene sequences into the host cell chromosome. Cells which have stably integrated the introduced DNA into their chromosomes can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector. The marker may complement an auxotrophy in the host (such as leu2, or ura3, which are common yeast auxotrophic markers), biocide resistance, e.g., antibiotics, or heavy metals, such as copper, or the like. The selectable marker gene can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection.

In a preferred embodiment, the introduced sequence will be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors may be employed for this purpose. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

For a mammalian host, several possible vector systems are available for expression. One class of vectors utilize DNA elements which provide autonomously replicating extra-chromosomal plasmids, derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, or SV40 virus. A second class of vectors include vaccinia virus expression vectors. A third class of vectors relies upon the integration of the desired gene sequences into the host chromosome. Cells which have stably integrated the introduced DNA into their chromosomes may be selected by also introducing one or more markers (e.g., an exogenous gene) which allow selection of host cells which contain the expression vector. The marker may provide for prototropy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals, such as copper or the like. The selectable marker gene can either be directly linked to the DNA sequences to be

expressed, or introduced into the same cell by co-transformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include splice signals, as well as transcription promoters, enhancers, and termination signals. The cDNA expression vectors incorporating such elements include those described by Okayama, H., Mol. Cell. Biol., 3:280 (1983), and others.

Once the vector or DNA sequence containing the construct has been prepared for expression, the DNA construct may be introduced (transformed) into an appropriate host. Various techniques may be employed, such as protoplast fusion, calcium phosphate precipitation, electroporation or other conventional techniques.

TRANSGENIC ANIMALS

In another embodiment, the present invention relates to transgenic animals having cells that coexpress human CD4 and CCR8. Such transgenic animals represent a model system for the study of HIV infection and the development of more effective anti-HIV therapeutics.

The term "animal" here denotes all mammalian species except human. It also includes an individual animal in all stages of development, including embryonic and fetal stages. Farm animals (pigs, goats, sheep, cows, horses, rabbits and the like), rodents (such as mice), and domestic pets (for example, cats and dogs) are included within the scope of the present invention.

A "transgenic" animal is any animal containing cells that bear genetic information received, directly or indirectly, by deliberate genetic manipulation at the subcellular level, such as by microinjection or infection with recombinant virus. "Transgenic" in the present context does not encompass classical crossbreeding or *in vitro* fertilization, but rather denotes animals in which one or more cells receive a recombinant DNA molecule. Although it is highly preferred that this molecule be integrated within the animal's

chromosomes, the present invention also contemplates the use of extrachromosomally replicating DNA sequences, such as might be engineered into yeast artificial chromosomes.

The term "transgenic animal" also includes a "germ cell line" transgenic animal. A germ cell line transgenic animal is a transgenic animal in which the genetic information has been taken up and incorporated into a germ line cell, therefore conferring the ability to transfer the information to offspring. If such offspring in fact possess some or all of that information, then they, too, are transgenic animals.

It is highly preferred that the transgenic animals of the present invention be produced by introducing into single cell embryos DNA encoding CCR8 and DNA encoding human CD4, in a manner such that these polynucleotides are stably integrated into the DNA of germ line cells of the mature animal and inherited in normal mendelian fashion. Advances in technologies for embryo micromanipulation now permit introduction of heterologous DNA into fertilized mammalian ova. For instance, totipotent or pluripotent stem cells can be transformed by microinjection, calcium phosphate mediated precipitation, liposome fusion, retroviral infection or other means, the transformed cells are then introduced into the embryo, and the embryo then develops into a transgenic animal. In a preferred method, developing embryos are infected with a retrovirus containing the desired DNA, and transgenic animals produced from the infected embryo.

In a most preferred method the appropriate DNAs are coinjected into the pronucleus or cytoplasm of embryos, preferably at the single cell stage, and the embryos allowed to develop into mature transgenic animals. These techniques are well known. For instance, reviews of standard laboratory procedures for microinjection of heterologous DNAs into mammalian (mouse, pig, rabbit, sheep, goat, cow) fertilized ova include: Hogan *et al.*, MANIPULATING THE MOUSE EMBRYO (Cold Spring Harbor Press 1986); Krimpenfort *et al.*, *Bio/Technology* 9:86 (1991); Palmiter *et al.*, *Cell* 41:343 (1985); Kraemer *et al.*, GENETIC MANIPULATION OF THE EARLY MAMMALIAN

EMBRYO (Cold Spring Harbor Laboratory Press 1985); Hammer *et al.*, *Nature*, 315:680 (1985); Purcel *et al.*, *Science*, 244:1281 (1986); Wagner *et al.*, U.S. patent No. 5,175,385; Krimpenfort *et al.*, U.S. patent No. 5,175,384, the respective contents of which are incorporated by reference.

The cDNA that encodes CCR8 can be fused in proper reading frame under the transcriptional and translational control of a vector to produce a genetic construct that is then amplified, for example, by preparation in a bacterial vector, according to conventional methods. See, for example, the standard work: Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL (Cold Spring Harbor Press 1989), the contents of which are incorporated by reference. The amplified construct is thereafter excised from the vector and purified for use in producing transgenic animals.

Production of transgenic animals containing the gene for human CD4 have been described. *See* Snyder *et al.*, *supra*; Dunn *et al.*, *supra*, the contents of which are incorporated by reference.

The term "transgenic" as used herein additionally includes any organism whose genome has been altered by *in vitro* manipulation of the early embryo or fertilized egg or by any transgenic technology to induce a specific gene knockout. The term "gene knockout@ as used herein, refers to the targeted disruption of a gene *in vivo* with complete loss of function that has been achieved by any transgenic technology familiar to those in the art. In one embodiment, transgenic animals having gene knockouts are those in which the target gene has been rendered nonfunctional by an insertion targeted to the gene to be rendered non-functional by homologous recombination. As used herein, the term "transgenic" includes any transgenic technology familiar to those in the art which can produce an organism carrying an introduced transgene or one in which an endogenous gene has been rendered non-functional or " knocked out."

The transgene to be used in the practice of the subject invention is a DNA sequence comprising a modified CCR8 coding sequence. In a preferred embodiment, the CCR8 gene is disrupted by homologous targeting in embryonic stem cells. For example, the entire mature C-terminal region of the CCR8 gene may be deleted as described in the examples below. Optionally, the CCR8 disruption or deletion may be accompanied by insertion of or replacement with other DNA sequences, such as a non-functional CCR8 sequence. In other embodiments, the transgene comprises DNA antisense to the coding sequence for CCR8. In another embodiment, the transgene comprises DNA encoding an antibody or receptor peptide sequence which is able to bind to CCR8. Where appropriate, DNA sequences that encode proteins having CCR8 activity but differ in nucleic acid sequence due to the degeneracy of the genetic code may also be used herein, as may truncated forms, allelic variants and interspecies homologues.

ANTIBODIES WHICH BIND TO CCR8 INHIBIT FUSION

In another embodiment, the present invention relates to antibodies that bind CCR8 that block *env*-mediated membrane fusion (i) associated with HIV entry into a human CD4-positive target cell or (ii) between an HIV-infected cell and an uninfected human CD4-positive target cell. The invention also includes antibodies that bind to CCR8 and inhibit chemokine binding. For example, such antibodies may be useful for ameliorating immune response disorders associated with macrophages. Antibodies of the invention may also inhibit gp120 binding to CCR8. Such antibodies could represent research and diagnostic tools in the study of HIV infection and the development of more effective anti-HIV therapeutics. In addition, pharmaceutical compositions comprising antibodies against CCR8 may represent effective anti-HIV therapeutics.

An antibody suitable for blocking *env*-mediated membrane fusion, inhibiting chemokine binding, or blocking gp120 binding to CCR8, is preferably specific for at least one portion of an extracellular region of the CCR8 polypeptide. For example, one of skill in the art can use extracellular amino acids of CCR8 to generate appropriate antibodies of

the invention. Alternatively, one of skill in the art can use whole cells expressing CCR8 as an immunogen for generation of anti-CCR8 antibodies which either block *env*-mediated membrane fusion, inhibit chemokine binding or block gp120 binding to CCR8. Anti-CCR8 antibodies of the invention may have any or all of these functions.

A target cell includes but is not limited to a cell of the following types: Mv 1 lu, NIH 3T3, BS-C-1, HEK293 cells and primary human T-cells and macrophages. Antibodies of the invention include polyclonal antibodies, monoclonal antibodies, and fragments of polyclonal and monoclonal antibodies.

The preparation of polyclonal antibodies is well-known to those skilled in the art. See, for example, Green *et al.*, *Production of Polyclonal Antisera*, in IMMUNOCHEMICAL PROTOCOLS (Manson, ed.), pages 1-5 (Humana Press 1992); Coligan *et al.*, *Production of Polyclonal Antisera in Rabbits, Rats, Mice and Hamsters*, in CURRENT PROTOCOLS IN IMMUNOLOGY, section 2.4.1 (1992), which are hereby incorporated by reference.

The preparation of monoclonal antibodies likewise is conventional. See, for example, Kohler & Milstein, *Nature* 256:495 (1975); Coligan *et al.*, sections 2.5.1-2.6.7; and Harlow *et al.*, ANTIBODIES: A LABORATORY MANUAL, page 726 (Cold Spring Harbor Pub. 1988), which are hereby incorporated by reference. Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures. Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. *See, e.g.*, Coligan *et al.*, sections 2.7.1-2.7.12 and sections

2.9.1-2.9.3; Barnes *et al.*, *Purification of Immunoglobulin G (IgG)*, in METHODS IN MOLECULAR BIOLOGY, VOL. 10, pages 79-104 (Humana Press 1992). Methods of *in vitro* and *in vivo* multiplication of monoclonal antibodies is well-known to those skilled in the art. Multiplication *in vitro* may be carried out in suitable culture media such as Dulbecco's Modified Eagle Medium or RPMI 1640 medium, optionally replenished by a mammalian serum such as fetal calf serum or trace elements and growth-sustaining supplements such as normal mouse peritoneal exudate cells, spleen cells, bone marrow macrophages. Production *in vitro* provides relatively pure antibody preparations and allows scale-up to yield large amounts of the desired antibodies. Large scale hybridoma cultivation can be carried out by homogenous suspension culture in an airlift reactor, in a continuous stirrer reactor, or in immobilized or entrapped cell culture. Multiplication *in vivo* may be carried out by injecting cell clones into mammals histocompatible with the parent cells, *e.g.*, osyngeneic mice, to cause growth of antibody-producing tumors. Optionally, the animals are primed with a hydrocarbon, especially oils such as pristane (tetramethylpentadecane) prior to injection. After one to three weeks, the desired monoclonal antibody is recovered from the body fluid of the animal.

Therapeutic applications for antibodies disclosed herein are also part of the present invention. For example, antibodies of the present invention may also be derived from subhuman primate antibody. General techniques for raising therapeutically useful antibodies in baboons can be found, for example, in Goldenberg *et al.*, International Patent Publication WO 91/11465 (1991) and Losman *et al.*, *Int. J. Cancer* 46:310 (1990), which are hereby incorporated by reference.

Alternatively, a therapeutically useful anti-CCR8 antibody may be derived from a "humanized" monoclonal antibody. Humanized monoclonal antibodies are produced by transferring mouse complementarity determining regions from heavy and light variable chains of the mouse immunoglobulin into a human variable domain, and then substituting human residues in the framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential

problems associated with the immunogenicity of murine constant regions. General techniques for cloning murine immunoglobulin variable domains are described, for example, by Orlandi *et al.*, *Proc. Nat'l Acad. Sci. USA* 86:3833 (1989), which is hereby incorporated in its entirety by reference. Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al.*, *Nature* 321: 522 (1986); Riechmann *et al.*, *Nature* 332: 323 (1988); Verhoeyen *et al.*, *Science* 239: 1534 (1988); Carter *et al.*, *Proc. Nat'l Acad. Sci. USA* 89: 4285 (1992); Sandhu, *Crit. Rev. Biotech.* 12: 437 (1992); and Singer *et al.*, *J. Immunol.* 150: 2844 (1993), which are hereby incorporated by reference.

Antibodies of the invention also may be derived from human antibody fragments isolated from a combinatorial immunoglobulin library. See, for example, Barbas *et al.*, METHODS: A COMPANION TO METHODS IN ENZYMOLOGY, VOL. 2, page 119 (1991); Winter *et al.*, *Ann. Rev. Immunol.* 12: 433 (1994), which are hereby incorporated by reference. Cloning and expression vectors that are useful for producing a human immunoglobulin phage library can be obtained, for example, from STRATAGENE Cloning Systems (La Jolla, CA).

In addition, antibodies of the present invention may be derived from a human monoclonal antibody. Such antibodies are obtained from transgenic mice that have been "engineered" to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain loci are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green *et al.*, *Nature Genet.* 7:13 (1994); Lonberg *et al.*, *Nature* 368:856 (1994); and Taylor *et al.*, *Int. Immunol.* 6:579 (1994), which are hereby incorporated by reference.

Antibody fragments of the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. patents No. 4,036,945 and No. 4,331,647, and references contained therein. These patents are hereby incorporated in their entireties by reference. *See also* Nisonhoff *et al.*, *Arch. Biochem. Biophys.* 89:230 (1960); Porter, *Biochem. J.* 73:119 (1959); Edelman *et al.*, METHODS IN ENZYMOLOGY, VOL. 1, page 422 (Academic Press 1967); and Coligan *et al.* at sections 2.8.1-2.8.10 and 2.10.1-2.10.4.

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

For example, Fv fragments comprise an association of V_H and V_L chains. This association may be noncovalent, as described in Inbar *et al.*, *Proc. Nat'l Acad. Sci. USA* 69:2659 (1972). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. *See, e.g.*, Sandhu, *supra*. Preferably, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as *E. coli*. The recombinant host cells

synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described, for example, by Whitlow *et al.*, METHODS: A COMPANION TO METHODS IN ENZYMOLOGY, VOL. 2, page 97 (1991); Bird *et al.*, *Science* 242:423-426 (1988); Ladner *et al.*, U.S. patent No. 4,946,778; Pack *et al.*, *Bio/Technology* 11: 1271-77 (1993); and Sandhu, *supra*.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells. See, for example, Larrick *et al.*, METHODS: A COMPANION TO METHODS IN ENZYMOLOGY, VOL. 2, page 106 (1991).

VARIANTS OF CCR8

The term "CCR8 variant" as used herein means a molecule that simulates at least part of the structure of CCR8 and interferes with the fusion of cells that express *env* with cells that express CD4 and CCR8. The *env* protein of certain HIV isolates may participate in HIV infectivity by binding to CCR8 at the surface of certain cells. CCR8 variants may also be useful in preventing chemokine binding, thereby ameliorating symptoms immune disorders.

PEPTIDE FRAGMENTS OF CCR8

Substantially purified peptide fragments of CCR8 that block membrane fusion between HIV and a target cell or cell fusion between an HIV-infected cell and a susceptible uninfected cell. A "susceptible" uninfected cell should express both CD4 and CCR8. Such peptide fragments could represent research and diagnostic tools in the study of HIV infection and the development of more effective anti-HIV therapeutics. In addition,

pharmaceutical compositions comprising isolated and purified peptide fragments of CCR8 may represent effective anti-HIV therapeutics.

The invention relates not only to fragments of naturally-occurring CCR8, but also to CCR8 mutants and chemically synthesized derivatives of CCR8 that block membrane fusion between HIV and a target cell.

In one embodiment, the present invention relates to peptides and peptide derivatives that have fewer amino acid residues than CCR8 and that block membrane fusion between HIV and a target cell. Such peptides and peptide derivatives could represent research and diagnostic tools in the study of HIV infection and the development of more effective anti-HIV therapeutics. The preferred peptide fragments of CCR8 according to the invention include those which correspond to the regions of CCR8 that are exposed on the cell surface.

The invention relates not only to peptides and peptide derivatives of naturally-occurring CCR8, but also to CCR8 mutants and chemically synthesized derivatives of CCR8 that block membrane fusion between HIV and a target cell. For example, changes in the amino acid sequence of CCR8 are contemplated in the present invention. CCR8 can be altered by changing the DNA encoding the protein. Preferably, only conservative amino acid alterations are undertaken, using amino acids that have the same or similar properties. Illustrative amino acid substitutions include the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine, glutamine, or glutamate; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; valine to isoleucine or leucine.

Additionally, other variants and fragments of CCR8 can be used in the present invention. Variants useful for the present invention comprise analogs, homologs, muteins and mimetics of CCR8 that retain the ability to block membrane fusion. Peptides of the CCR8 refer to portions of the amino acid sequence of CCR8 that also retain this ability. The variants can be generated directly from CCR8 itself by chemical modification, by proteolytic enzyme digestion, or by combinations thereof. Additionally, genetic engineering techniques, as well as methods of synthesizing polypeptides directly from amino acid residues, can be employed.

Peptides of the invention can be synthesized by such commonly used methods as t-BOC or FMOC protection of alpha-amino groups. Both methods involve stepwise syntheses whereby a single amino acid is added at each step starting from the C terminus of the peptide (See, Coligan, *et al., Current Protocols in Immunology,* Wiley Interscience, 1991, Unit 9). Peptides of the invention can also be synthesized by the well known solid phase peptide synthesis methods described Merrifield, *J. Am. Chem. Soc.*, 85:2149, 1962), and Stewart and Young, *Solid Phase Peptides Synthesis*, (Freeman, San Francisco, 1969, pp.27-62), using a copoly(styrene-divinylbenzene) containing 0.1-1.0 mMol amines/g polymer. On completion of chemical synthesis, the peptides can be deprotected and cleaved from the polymer by treatment with liquid HF-10% anisole for about 1/4-1 hours at 0°C. After evaporation of the reagents, the peptides are extracted from the polymer with 1% acetic acid solution which is then lyophilized to yield the crude material. This can normally be purified by such techniques as gel filtration on Sephadex G-15 using 5% acetic acid as a solvent. Lyophilization of appropriate fractions of the column will yield the homogeneous peptide or peptide derivatives, which can then be characterized by such standard techniques as amino acid analysis, thin layer chromatography, high performance liquid chromatography, ultraviolet absorption spectroscopy, molar rotation, solubility, and quantitated by the solid phase Edman degradation.

Alternatively, peptides can be produced by recombinant methods as described below.

The term "substantially purified" as used herein refers to a molecule, such as a peptide that is substantially free of other proteins, lipids, carbohydrates, nucleic acids, and other biological materials with which it is naturally associated. For example, a substantially pure molecule, such as a polypeptide, can be at least 60%, by dry weight, the molecule of interest. One skilled in the art can purify CCR8 peptides using standard protein purification methods and the purity of the polypeptides can be determined using standard methods including, *e.g.*, polyacrylamide gel electrophoresis (*e.g.*, SDS-PAGE), column chromatography (*e.g.*, high performance liquid chromatography (HPLC)), and amino-terminal amino acid sequence analysis.

Non-peptide compounds that mimic the binding and function of CCR8 ("mimetics") can be produced by the approach outlined in Saragovi *et al.*, *Science* 253: 792-95 (1991). Mimetics are molecules which mimic elements of protein secondary structure. See, for example, Johnson *et al.*,"Peptide Turn Mimetics," in BIOTECHNOLOGY AND PHARMACY, Pezzuto *et al.*, Eds., (Chapman and Hall, New York 1993). The underlying rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions. For the purposes of the present invention, appropriate mimetics can be considered to be the equivalent of CCR8 itself.

Longer peptides can be produced by the "native chemical" ligation technique which links together peptides (Dawson, *et al., Science*, 266:776, 1994). Variants can be created by recombinant techniques employing genomic or cDNA cloning methods. Site-specific and region-directed mutagenesis techniques can be employed. See CURRENT PROTOCOLS IN MOLECULAR BIOLOGY vol. 1, ch. 8 (Ausubel *et al.* eds., J. Wiley & Sons 1989 & Supp. 1990-93); PROTEIN ENGINEERING (Oxender & Fox eds., A. Liss, Inc. 1987). In addition, linker-scanning and PCR-mediated techniques can be employed for mutagenesis. See PCR TECHNOLOGY (Erlich ed., Stockton Press 1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, vols. 1 & 2, *supra*. Protein sequencing, structure and modeling approaches for use with any of the above techniques

are disclosed in PROTEIN ENGINEERING, *loc. cit.*, and CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, vols. 1 & 2, *supra.*

If the compounds described above are employed, the skilled artisan can routinely insure that such compounds are amenable for use with the present invention utilizing cell fusion assays known in the art, or for example, the vaccinia cell fusion system described herein. If a compound blocks *env*-mediated membrane fusion (I) involved in HIV entry into a human CD4-positive target cell or (ii) between an HIV-infected cell and an uninfected human CD4-positive target cell, the compounds are suitable according to the invention. The preferred peptide fragments of CCR8 according to the invention include those which correspond to the regions of CCR8 that are exposed on the cell surface.

CCR8-BINDING AND BLOCKING AGENTS

In yet another embodiment, the present invention relates to CCR8-binding agents that block membrane fusion between HIV and a target cell. Such agents could represent research and diagnostic tools in the study of HIV infection and the development of more effective anti-HIV therapeutics. In addition, pharmaceutical compositions comprising CCR8-binding agents may represent effective anti-HIV therapeutics. In the context of HIV infection, the phrase "CCR8-binding agent" denotes a naturally occurring ligand of CCR8 such as, for example: I-309; a synthetic ligand of CCR8, or appropriate derivatives of the natural or synthetic ligands. Various chemokines may function as a binding biologic agent as a ligand for CCR8. For example, I-309 is a ligand for CCR8 and is included as a biologic agent of the invention. Derivatives, analogs, mutants and CCR8 binding fragments of I-309 are useful for blocking *env*-mediated membrane fusion. The determination and isolation of ligands is well described in the art. *See, e.g.*, Lerner, *Trends NeuroSci.* 17:142-146 (1994) which is hereby incorporated in its entirety by reference. A CCR8-binding agent that blocks *env*-mediated membrane fusion (i) involved in HIV entry into a human CD4-positive target cell or (ii) between an HIV-infected cell and an uninfected human CD4-positive target cell is suitable according

to the invention. Further, a CCR8 blocking or binding agent includes an agent which inhibits gp120 binding to CCR8 or chemokine binding to CCR8.

In yet another embodiment, the present invention relates to CCR8-binding agents that interfere with binding between CCR8 and a chemokine. Such binding agents may interfere by competitive inhibition, by non-competitive inhibition or by uncompetitive inhibition. Interference with normal binding between CCR8 and one or more chemokines can result in a useful pharmacological effect related to inflammation because CCR8 binds chemokines that regulate monocyte accumulation and activation in inflamed tissue sites. Monocytes are long-lived cells capable of further differentiation as they move from the blood to establish residence in the tissues as macrophages. The functional properties of tissue macrophages differ in different organs, and in the same organ depending on the presence of priming agents, *i.e.*, agents that can change the behavior of monocytes and make them more sensitive to chemoattractants. CCR8-binding or blocking agents can interfere with the normal functioning of this system to reduce inflammation and are contemplated by the present invention. Anti-CCR8 antibodies of the invention are also useful in this context.

SCREEN FOR CCR5 BINDING AND BLOCKING COMPOSITIONS

In another embodiment, the invention provides a method for identifying a composition which binds to CCR8 or blocks HIV *env*-mediated membrane fusion. The method includes incubating components comprising the composition and CCR8 under conditions sufficient to allow the components to interact and measuring the binding of the composition to CCR8. Compositions that bind to CCR8 include peptides, peptidomimetics, polypeptides, chemical compounds and biologic agents as described above. In addition to inhibition of cell fusion, one of skill in the art could screen for inhibition of gp120 binding or inhibition of CCR8 binding to a chemokine to determine if a compound or composition was a CCR8 binding or blocking agent.

Incubating includes conditions which allow contact between the test composition and CCR8. Contacting includes in solution and in solid phase. The test ligand(s)/composition may optionally be a combinatorial library for screening a plurality of compositions. Compositions identified in the method of the invention can be further evaluated, detected, cloned, sequenced, and the like, either in solution or after binding to a solid support, by any method usually applied to the detection of a specific DNA sequence such as PCR, oligomer restriction (Saiki, *et al., Bio/Technology*, 3:1008-1012, 1985), allele-specific oligonucleotide (ASO) probe analysis (Conner, *et al., Proc. Natl. Acad. Sci. USA*, 80:278, 1983), oligonucleotide ligation assays (OLAs) (Landegren, *et al., Science*, 241:1077, 1988), and the like. Molecular techniques for DNA analysis have been reviewed (Landegren, *et al., Science*, 242:229-237, 1988).

To determine if a composition can functionally complex with the receptor protein, induction of the exogenous gene is monitored by monitoring changes in the protein levels of the protein encoded for by the exogenous gene, for example. When a composition(s) is found that can induce transcription of the exogenous gene, it is concluded that this composition(s) can bind to the receptor protein coded for by the nucleic acid encoding the initial sample test composition(s).

Expression of the exogenous gene can be monitored by a functional assay or assay for a protein product, for example. The exogenous gene is therefore a gene which will provide an assayable/measurable expression product in order to allow detection of expression of the exogenous gene. Such exogenous genes include, but are not limited to, reporter genes such as chloramphenicol acetyltransferase gene, an alkaline phosphatase gene, beta-galactosidase,a luciferase gene, a green fluorescent protein gene, guanine xanthine phosphoribosyltransferase, alkaline phosphatase, and antibiotic resistance genes (e.g., neomycin phosphotransferase).

Expression of the exogenous gene is indicative of composition-receptor binding, thus, the binding or blocking composition can be identified and isolated. The compositions

of the present invention can be extracted and purified from the culture media or a cell by using known protein purification techniques commonly employed, such as extraction, precipitation, ion exchange chromatography, affinity chromatography, gel filtration and the like. Compositions can be isolated by affinity chromatography using the modified receptor protein extracellular domain bound to a column matrix or by heparin chromatography.

Also included in the screening method of the invention is combinatorial chemistry methods for identifying chemical compounds that bind to CCR8. Ligands/compositions that bind to CCR8 can be assayed in standard cell:cell fusion assays, such as the vaccinia assay described herein to determine whether the composition inhibits or blocks *env*-mediated membrane fusion (i) involved in HIV entry into a human CD4-positive target cell or (ii) between an HIV-infected cell and an uninfected human CD4-positive target cell. Screening methods include inhibition of chemokine binding to CCR8 (*e.g.*, use radiolabeled chemokine) or inhibition of labeled gp120. For example, a derivative of RANTES was shown to act as a CCR5 receptor antagonist (RANTES 9-68; Arenzana-Selsdedos et al., *Nature* 383:400, 1996, incorporated by reference). AOP-RANTES and Met-RANTES were shown to bind with high affinity yet failed to induce chemotaxis signalling, thereby acting as an antagonist (Simmons et al., *Science* 276:276, 1997). Thus, the screening method is also useful for identifying variants, binding or blocking agents, etc., which functionally, if not physically (*e.g.*, sterically) act as antagonists or agonists, as desired.

PHARMACEUTICAL COMPOSITIONS

The invention also includes various pharmaceutical compositions that block membrane fusion between HIV and a target cell. The pharmaceutical compositions according to the invention are prepared by bringing an antibody against CCR8, a peptide or peptide derivative of CCR8, a CCR8 mimetic, or a CCR8-binding agent according to the present invention into a form suitable for administration to a subject using carriers, excipients

and additives or auxiliaries. Frequently used carriers or auxiliaries include magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch, vitamins, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols and solvents, such as sterile water, alcohols, glycerol and polyhydric alcohols. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial, anti-oxidants, chelating agents and inert gases. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described, for instance, in *Remington's Pharmaceutical Sciences*, 15th ed. Easton: Mack Publishing Co., 1405-1412, 1461-1487 (1975) and *The National Formulary XIV.*, 14th ed. Washington: American Pharmaceutical Association (1975), the contents of which are hereby incorporated by reference. The pH and exact concentration of the various components of the pharmaceutical composition are adjusted according to routine skills in the art. *See Goodman and Gilman's The Pharmacological Basis for Therapeutics* (7th ed.).

In another embodiment, the invention relates to a method of blocking the membrane fusion between HIV and a target cell. This method involves administering to a subject a therapeutically effective dose of a pharmaceutical composition containing the compounds of the present invention and a pharmaceutically acceptable carrier. "Administering" the pharmaceutical composition of the present invention may be accomplished by any means known to the skilled artisan. By "subject" is meant any mammal, preferably a human.

The pharmaceutical compositions are preferably prepared and administered in dose units. Solid dose units are tablets, capsules and suppositories. For treatment of a patient, depending on activity of the compound, manner of administration, nature and severity of the disorder, age and body weight of the patient, different daily doses are necessary. Under certain circumstances, however, higher or lower daily doses may be appropriate. The administration of the daily dose can be carried out both by single administration in

the form of an individual dose unit or else several smaller dose units and also by multiple administration of subdivided doses at specific intervals.

The pharmaceutical compositions according to the invention are in general administered topically, intravenously, orally or parenterally or as implants, but even rectal use is possible in principle. Suitable solid or liquid pharmaceutical preparation forms are, for example, granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, aerosols, drops or injectable solution in ampule form and also preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of present methods for drug delivery, see Langer, *Science*, 249: 1527-1533 (1990), which is incorporated herein by reference.

The pharmaceutical compositions according to the invention may be administered locally or systemically. By "therapeutically effective dose" is meant the quantity of a compound according to the invention necessary to prevent, to cure or at least partially arrest the symptoms of the disease and its complications. Amounts effective for this use will, of course, depend on the severity of the disease and the weight and general state of the patient. Typically, dosages used *in vitro* may provide useful guidance in the amounts useful for *in situ* administration of the pharmaceutical composition, and animal models may be used to determine effective dosages for treatment of particular disorders. Various considerations are described, *e.g.*, in Gilman *et al.* (eds.) (1990) GOODMAN AND GILMAN'S: THE PHARMACOLOGICAL BASES OF THERAPEUTICS, 8th ed., Pergamon Press; and REMINGTON'S PHARMACEUTICAL SCIENCES, 17th ed. (1990), Mack Publishing Co., Easton, Pa., each of which is herein incorporated by reference.

TESTING FOR NEW PHARMACEUTICAL COMPOSITIONS

In a preferred embodiment, the invention is a method for screening a compound ("test substance") for anti-HIV pharmacological activity. In this embodiment, the CCR8 and CD4 genes are expressed in one type of eukaryotic cell and incubated with a second type of eukaryotic cell that expresses an HIV envelope protein ("*env*"). Fusion between at least one cell of each type with the other type is then monitored. The test substance is added to the incubation solution before or after mixing of the cells and its effect on the fusion rate of cells is determined by any of a number of means. One means to monitor fusion is to include a system that results in the production of an active β-galactosidase upon cell fusion as described in Nussbaum et al., 1994, *supra*. If the test molecule inhibits HIV infectivity then the presence of the molecule will decrease the cell fusion response. In the case where the test substance binds a naturally occurring molecule present in the human that is necessary for HIV infectivity, then addition of the test molecule may decrease cell fusion.

The cell fusion assay can be used to determine the functional ability of CCR8 to confer *env*-mediated fusion competence to a diverse range of CD4-positive (*e.g.*, either recombinantly produced or naturally occurring) cell types: *e.g.*, NIH 3T3 (murine); BS-C-1 (African green monkey); HEK293 (human); and Mv 1 Lu (mink). In addition, unusual, fusion-incompetent, CD4-positive human cell types can be employed (U-87 MG glioblastoma; and SCL1).

Variations of drug screening methods are known to the artisan of average skill in this field. Consequently, the cell fusion assay can be used in a wide variety of formats to exploit the properties of the CCR8 receptor to screen for drugs that are effective against HIV.

ANTISENSE or RIBOZYME INHIBITION OF CCR8 FOR HIV THERAPY

Antisense technology offers a very specific and potent means of inhibiting HIV infection of cells that contain CCR8, for example, by decreasing the amount of CCR8 expression in a cell. Antisense polynucleotides in context of the present invention includes both short sequences of DNA known as oligonucleotides of usually 10-50 bases in length as well as longer sequences of DNA that may exceed the length of the CCR8 gene sequence itself. Antisense polynucleotides useful for the present invention are complementary to specific regions of a corresponding target mRNA. Hybridization of antisense polynucleotides to their target transcripts can be highly specific as a result of complementary base pairing. The capability of antisense polynucleotides to hybridize is affected by such parameters as length, chemical modification and secondary structure of the transcript which can influence polynucleotide access to the target site. See Stein *et al*, *Cancer Research* 48:2659 (1988). An antisense polynucleotide can be introduced to a cell by introducing a DNA segment that codes for the polynucleotide into the cell such that the polynucleotide is made inside the cell. An antisense polynucleotide can also be introduced to a cell by adding the polynucleotide to the environment of the cell such that the cell can take up the polynucleotide directly. The latter route is preferred for the shorter polynucleotides of up to about 20 bases in length.

In selecting the preferred length for a given polynucleotide, a balance must be struck to gain the most favorable characteristics. Shorter polynucleotides such as 10-to 15-mers, while offering higher cell penetration, have lower gene specificity. In contrast, while longer polynucleotides of 20-30 bases offer better specificity, they show decreased uptake kinetics into cells. See Stein *et al.*, PHOSPHOROTHIOATE OLIGODEOXYNUCLEOTIDE ANALOGUES in "Oligodeoxynucleotides - Antisense Inhibitors of Gene Expression" Cohen, ed. McMillan Press, London (1988). Accessibility to mRNA target sequences also is of importance and, therefore, loop-forming regions in targeted mRNAs offer promising targets. In this disclosure the term "polynucleotide" encompasses both oligomeric nucleic acid moieties of the type found

in nature, such as the deoxyribonucleotide and ribonucleotide structures of DNA and RNA, and man-made analogues which are capable of binding to nucleic acids found in nature. The polynucleotides of the present invention can be based upon ribonucleotide or deoxyribonucleotide monomers linked by phosphodiester bonds, or by analogues linked by methyl phosphonate, phosphorothioate, or other bonds. They may also comprise monomer moieties which have altered base structures or other modifications, but which still retain the ability to bind to naturally occurring DNA and RNA structures. Such polynucleotides may be prepared by methods well-known in the art, for instance using commercially available machines and reagents available from Perkin-Elmer/Applied Biosystems (Foster City, CA).

Phosphodiester-linked polynucleotides are particularly susceptible to the action of nucleases in serum or inside cells, and therefore in a preferred embodiment the polynucleotides of the present invention are phosphorothioate or methyl phosphonate-linked analogues, which have been shown to be nuclease-resistant. Persons of ordinary skill in this art will be able to select other linkages for use in the invention. These modifications also may be designed to improve the cellular uptake and stability of the polynucleotides.

In another embodiment of the invention, the antisense polynucleotide is an RNA molecule produced by introducing an expression construct into the target cell. The RNA molecule thus produced is chosen to have the capability to hybridize to CCR8 mRNA. Such molecules that have this capability can inhibit translation of the CCR8 mRNA and thereby inhibit the ability of HIV to infect cells that contain the RNA molecule.

The polynucleotides which have the capability to hybridize with mRNA targets can inhibit expression of corresponding gene products by multiple mechanisms. In "translation arrest," the interaction of polynucleotides with target mRNA blocks the action of the ribosomal complex and, hence, prevents translation of the messenger RNA into protein. Haeuptle *et al.*, *Nucl. Acids. Res.* 14:1427 (1986). In the case of

phosphodiester or phosphorothioate DNA polynucleotides, intracellular RNase H can digest the targeted RNA sequence once it has hybridized to the DNA oligomer. Walder and Walder, *Proc. Natl. Acad. Sci. USA* 85:5011 (1988). As a further mechanism of action, in "transcription arrest" it appears that some polynucleotides can form "triplex," or triple-helical structures with double stranded genomic DNA containing the gene of interest, thus interfering with transcription by RNA polymerase. Giovannangeli *et al.*, *Proc. Natl. Acad. Sci.* 90:10013 (1993); Ebbinghaus *et al. J. Clin. Invest.* 92:2433 (1993).

In one embodiment, CCR8 polynucleotides are synthesized according to standard methodology. Phosphorothioate modified DNA polynucleotides typically are synthesized on automated DNA synthesizers available from a variety of manufacturers. These instruments are capable of synthesizing nanomole amounts of polynucleotides as long as 100 nucleotides. Shorter polynucleotides synthesized by modern instruments are often suitable for use without further purification. If necessary, polynucleotides may be purified by polyacrylamide gel electrophoresis or reverse phase chromatography. See Sambrook *et al.*, *MOLECULAR CLONING: A Laboratory Manual*, Vol. 2, Chapter 11, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989).

Alternatively, a CCR8 polynucleotide in the form of antisense RNA may be introduced to a cell by its expression within the cell from a standard DNA expression vector. CCR8 DNA antisense sequences can be cloned from standard plasmids into expression vectors, which expression vectors have characteristics permitting higher levels of, or more efficient expression of the resident polynucleotides. At a minimum, these constructs require a prokaryotic or eukaryotic promoter sequence which initiates transcription of the inserted DNA sequences. A preferred expression vector is one where the expression is inducible to high levels. This is accomplished by the addition of a regulatory region which provides increased transcription of downstream sequences in the appropriate host cell. See Sambrook *et al.*, Vol. 3, Chapter 16 (1989).

For example, CCR8 antisense expression vectors can be constructed using the polymerase chain reaction (PCR) to amplify appropriate fragments from single-stranded cDNA of a plasmid such as pRc in which CCR8 cDNA has been incorporated. Fang *et al., J. Biol. Chem.* 267 25889-25897 (1992). Polynucleotide synthesis and purification techniques are described in Sambrook *et al.* and Ausubel *et al.* (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Wiley Interscience 1987) (hereafter "Ausubel"), respectively. The PCR procedure is performed via well-known methodology. See, for example, Ausubel, and Bangham, "The Polymerase Chain Reaction: Getting Started," *in* PROTOCOLS IN HUMAN MOLECULAR GENETICS (Humana Press 1991). Moreover, PCR kits can be purchased from companies such as Stratagene Cloning Systems (La Jolla, CA) and Invitrogen (San Diego, CA).

The products of PCR are subcloned into cloning vectors. In this context, a "cloning vector" is a DNA molecule, such as a plasmid, cosmid or bacteriophage, that can replicate autonomously in a host prokaryotic cell. Cloning vectors typically contain one or a small number of restriction endonuclease recognition sites at which foreign DNA sequences can be inserted in a determinable fashion without loss of an essential biological function of the vector, as well as a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Suitable cloning vectors are described by Sambrook *et al.*, Ausubel, and Brown (ed.), MOLECULAR BIOLOGY LABFAX (Academic Press 1991). Cloning vectors can be obtained, for example, from GIBCO/BRL (Gaithersburg, MD), Clontech Laboratories, Inc. (Palo Alto, CA), Promega Corporation (Madison, WI), Stratagene Cloning Systems (La Jolla, CA), Invitrogen (San Diego, CA), and the American Type Culture Collection (Rockville, MD).

Preferably, the PCR products are ligated into a "TA" cloning vector. Methods for generating PCR products with a thymidine or adenine overhang are well-known to those of skill in the art. See, for example, Ausubel at pages 15.7.1-15.7.6. Moreover, kits for performing TA cloning can be purchased from companies such as Invitrogen (San Diego, CA).

Cloned antisense fragments are amplified by transforming competent bacterial cells with a cloning vector and growing the bacterial host cells in the presence of the appropriate antibiotic. See, for example, Sambrook *et al.*, and Ausubel. PCR is then used to screen bacterial host cells for CCR8 antisense orientation clones. The use of PCR for bacterial host cells is described, for example, by Hofmann *et al.*, "Sequencing DNA Amplified Directly from a Bacterial Colony," in PCR PROTOCOLS: METHODS AND APPLICATIONS, White (ed.), pages 205-210 (Humana Press 1993), and by Cooper *et al.*, "PCR-Based Full-Length cDNA Cloning Utilizing the Universal-Adaptor/Specific DOS Primer-Pair Strategy," *Id.* at pages 305-316.

Cloned antisense fragments are cleaved from the cloning vector and inserted into an expression vector. For example, *Hind*III and *Xba*I can be used to cleave the antisense fragment from TA cloning vector pCR™-II (Invitrogen;San Diego, CA). Suitable expression vectors typically contain (1) prokaryotic DNA elements coding for a bacterial origin of replication and an antibiotic resistance marker to provide for the amplification and selection of the expression vector in a bacterial host; (2) DNA elements that control initiation of transcription, such as a promoter; and (3) DNA elements that control the processing of transcripts, such as a transcription termination/polyadenylation sequence.

For a mammalian host, the transcriptional and translational regulatory signals preferably are derived from viral sources, such as adenovirus, bovine papilloma virus, simian virus, or the like, in which the regulatory signals are associated with a particular gene which has a high level of expression. Suitable transcriptional and translational regulatory sequences also can be obtained from mammalian genes, such as actin, collagen, myosin, and metallothionein genes.

Transcriptional regulatory sequences include a promoter region sufficient to direct the initiation of RNA synthesis. Suitable eukaryotic promoters include the promoter of the mouse metallothionein I gene (Hamer *et al.*, *J. Molec. Appl. Genet.* 1: 273 (1982)); the TK promoter of Herpes virus (McKnight, *Cell* 31: 355 (1982)); the SV40 early promoter

(Benoist *et al.*, *Nature* 290: 304 (1981); the Rous sarcoma virus promoter (Gorman *et al.*, *Proc. Nat'l Acad. Sci. USA* 79: 6777 (1982)); and the cytomegalovirus promoter (Foecking *et al.*, *Gene* 45: 101 (1980)).

Alternatively, a prokaryotic promoter, such as the bacteriophage T3 RNA polymerase promoter, can be used to control fusion gene expression if the prokaryotic promoter is regulated by a eukaryotic promoter. Zhou *et al.*, *Mol. Cell. Biol.* 10: 4529 (1990); Kaufman *et al.*, *Nucl. Acids Res.* 19: 4485 (1991).

A vector for introducing at least one antisense polynucleotide into a cell by expression from a DNA is the vector pRc/CMV (Invitrogen (San Diego, CA), which provides a high level of constitutive transcription from mammalian enhancer-promoter sequences. Cloned CCR8 antisense vectors are amplified in bacterial host cells, isolated from the cells, and analyzed as described above.

Another possible method by which antisense sequences may be exploited is via gene therapy. Virus-like vectors, usually derived from retroviruses, may prove useful as vehicles for the importation and expression of antisense constructs in human cells. Generally, such vectors are non-replicative *in vivo*, precluding any unintended infection of non-target cells. In such cases, helper cell lines are provided which supply the missing replicative functions *in vitro*, thereby permitting amplification and packaging of the antisense vector. A further precaution against accidental infection of non-target cells involves the use of target cell-specific regulatory sequences. When under the control of such sequences, antisense constructs would not be expressed in normal tissues.

Two prior studies have explored the feasibility of using antisense polynucleotides to inhibit the expression of a heparin binding growth factor. Kouhara *et al.*, *Oncogene* 9: 455-462 (1994); Morrison, *J. Biol. Chem.* 266: 728 (1991). Kouhara *et al.* showed that androgen-dependent growth of mouse mammary carcinoma cells (SC-3) is mediated through induction of androgen-induced, heparin binding growth factor (AIGF). An

antisense 15-mer corresponding to the translation initiation site of AIGF was measured for its ability to interfere with androgen-induction of SC-3 cells. At concentrations of 5 μM, the antisense polynucleotide effectively inhibited androgen-induced DNA synthesis. Morrison showed that antisense polynucleotides targeted against basic fibroblast growth factor can inhibit growth of astrocytes in culture. Thus, the general feasibility of targeting an individual gene product in a mammalian cell has been established.

Antisense polynucleotides according to the present invention are derived from any portion of the open reading frame of the CCR8 cDNA. Preferably, mRNA sequences (i) surrounding the translation initiation site and (ii) forming loop structures are targeted. Based upon the size of the human genome, statistical studies show that a DNA segment approximately 14-15 base pairs long will have a unique sequence in the genome. To ensure specificity of targeting CCR8 RNA, therefore, it is preferred that the antisense polynucleotides are at least 15 nucleotides in length. Thus, the shortest polynucleotides contemplated by the present invention encompass nucleotides corresponding to positions 1-14, 1-15, 1-16, 1-17, 1-18, 1-19, 2-16, 3-17, *etc.* of the CCR8 cDNA sequence. Position 1 refers to the first nucleotide of the CCR8 coding region.

Not every antisense polynucleotide will provide a sufficient degree of inhibition or a sufficient level of specificity for the CCR8 target. Thus, it will be necessary to screen polynucleotides to determine which have the proper antisense characteristics. A preferred method to assay for a useful antisense polynucleotide is the inhibition of cell fusion between: (1) cells that contain CD4 and CCR8; and (2) cells that contain *env*.

Administration of an antisense polynucleotide to a subject, either as a naked, synthetic polynucleotide or as part of an expression vector, can be effected via any common route (oral, nasal, buccal, rectal, vaginal, or topical), or by subcutaneous, intramuscular, intraperitoneal, or intravenous injection. Pharmaceutical compositions of the present invention, however, are advantageously administered in the form of injectable

compositions. A typical composition for such purpose comprises a pharmaceutically acceptable solvent or diluent and other suitable, physiologic compounds. For instance, the composition may contain polynucleotide and about 10 mg of human serum albumin per milliliter of a phosphate buffer containing NaCl.

As much as 700 milligrams of antisense polynucleotide has been administered intravenously to a patient over a course of 10 days (*i.e.*, 0.05 mg/kg/hour) without signs of toxicity. Sterling, "Systemic Antisense Treatment Reported," *Genetic Engineering News* 12: 1, 28 (1992).

Other pharmaceutically acceptable excipients include non-aqueous or aqueous solutions and non-toxic compositions including salts, preservatives, buffers and the like. Examples of non-aqueous solutions are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyloleate. Aqueous solutions include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, *etc.* Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components the pharmaceutical composition are adjusted according to routine skills in the art. A preferred pharmaceutical composition for topical administration is a dermal cream or transdermal patch.

Antisense polynucleotides or their expression vectors may be administered by injection as an oily suspension. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides. Moreover, antisense polynucleotides or vectors may be combined with a lipophilic carrier such as any one of a number of sterols including cholesterol, cholate and deoxycholic acid. A preferred sterol is cholesterol. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium

carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension also contains stabilizers.

An alternative formulation for the administration of antisense CCR8 polynucleotides involves liposomes. Liposome encapsulation provides an alternative formulation for the administration of antisense CCR8 polynucleotides and expression vectors. Liposomes are microscopic vesicles that consist of one or more lipid bilayers surrounding aqueous compartments. See, generally, Bakker-Woudenberg *et al., Eur. J. Clin. Microbiol. Infect. Dis.* 12 (Suppl. 1): S61 (1993), and Kim, *Drugs* 46: 618 (1993). Liposomes are similar in composition to cellular membranes and as a result, liposomes can be administered safely and are biodegradable. Depending on the method of preparation, liposomes may be unilamellar or multilamellar, and liposomes can vary in size with diameters ranging from 0.02 μm to greater than 10 μm. A variety of agents can be encapsulated in liposomes: hydrophobic agents partition in the bilayers and hydrophilic agents partition within the inner aqueous space(s). See, for example, Machy *et al.*, LIPOSOMES IN CELL BIOLOGY AND PHARMACOLOGY (John Libbey 1987), and Ostro *et al., American J. Hosp. Pharm.* 46: 1576 (1989). Moreover, it is possible to control the therapeutic availability of the encapsulated agent by varying liposome size, the number of bilayers, lipid composition, as well as the charge and surface characteristics of the liposomes.

Liposomes can adsorb to virtually any type of cell and then slowly release the encapsulated agent. Alternatively, an absorbed liposome may be endocytosed by cells that are phagocytic. Endocytosis is followed by intralysosomal degradation of liposomal lipids and release of the encapsulated agents. Scherphof *et al., Ann. N.Y. Acad. Sci.* 446: 368 (1985).

After intravenous administration, conventional liposomes are preferentially phagocytosed into the reticuloendothelial system. However, the reticuloendothelial system can be circumvented by several methods including saturation with large doses of liposome

particles, or selective macrophage inactivation by pharmacological means. Claassen *et al., Biochim. Biophys. Acta* 802: 428 (1984). In addition, incorporation of glycolipid- or polyethelene glycol-derivatised phospholipids into liposome membranes has been shown to result in a significantly reduced uptake by the reticuloendothelial system. Allen *et al., Biochim. Biophys. Acta* 1068: 133 (1991); Allen *et al., Biochim. Biohys. Acta* 1150: 9 (1993) These Stealth® liposomes have an increased circulation time and an improved targeting to tumors in animals. Woodle *et al., Proc. Amer. Assoc. Cancer Res.* 33: 2672 (1992). Human clinical trials are in progress, including Phase III clinical trials against Kaposi's sarcoma. Gregoriadis *et al., Drugs* 45: 15 (1993).

Antisense polynucleotides and expression vectors can be encapsulated within liposomes using standard techniques. A variety of different liposome compositions and methods for synthesis are known to those of skill in the art. See, for example, U.S. Patent No. 4,844,904, U.S. Patent No. 5,000,959, U.S. Patent No. 4,863,740, and U.S. Patent No. 4,975,282, all of which are hereby incorporated by reference.

Liposomes can be prepared for targeting to particular cells or organs by varying phospholipid composition or by inserting receptors or ligands into the liposomes. For instance, antibodies specific to tumor associated antigens may be incorporated into liposomes, together with antisense polynucleotides or expression vectors, to target the liposome more effectively to the tumor cells. See, for example, Zelphati *et al., Antisense Research and Development* 3: 323-338 (1993), describing the use "immunoliposomes" containing antisense polynucleotides for human therapy.

In general, the dosage of administered liposome-encapsulated antisense polynucleotides and vectors will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition and previous medical history. Dose ranges for particular formulations can be determined by using a suitable animal model.

The above approaches can also be used not only with antisense nucleic acid, but also with ribozymes, or triplex agents to block transcription or translation of a specific CCR8 mRNA, either by masking that mRNA with an antisense nucleic acid or triplex agent, or by cleaving it with a ribozyme.

Use of an oligonucleotide to stall transcription is known as the triplex strategy since the oligomer winds around double-helical DNA, forming a three-strand helix. Therefore, these triplex compounds can be designed to recognize a unique site on a chosen gene (Maher, *et al., Antisense Res. and Dev.*, 1(3):227, 1991; Helene, C., *Anticancer Drug Design*, 6(6):569, 1991).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, *J.Amer.Med. Assn.*, 260:3030, 1988). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

There are two basic types of ribozymes namely, *tetrahymena*-type (Hasselhoff, *Nature*, 334:585, 1988) and "hammerhead"-type. *Tetrahymena*-type ribozymes recognize sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type ribozymes for inactivating a specific mRNA species and 18-based recognition sequences are preferable to shorter recognition sequences.

HOMOZYGOUS AND HETEROZYGOUS MUTATIONS IN CCR8

It is known that in some cases, a homozygous or heterozygous mutation in a polypeptide or a regulatory region of a gene confers a molecular basis for a difference in function. Bertina, *et al.* and Greengard, *et al.* (Bertina, *et al., Nature*, 369:64, 1994; Greengard, *et al., Lancet*, 343:1361, 1994), first identified the molecular basis for the FV abnormality. The phenotype of APC resistance was shown to be associated with heterozygosity or homozygosity for a single point mutation in the FV gene that resulted in the substitution of arginine at amino acid residue 506 with glutamine (FV R506Q). This R506Q mutation prevents APC from cleaving a peptide bond at Arg-506 in FV that is required to inactivate factor Va (Bertina, *supra*; Sun, *et al., Blood*, 83:3120, 1994).

Similarly, the present invention envisions diagnostic and prognostic, and in addition, therapeutic approaches to treatment of HIV-associated syndromes based on homozygosity or heterozygosity of CCR8 mutants. For example, while not wanting to be bound by a particular theory, it is believed that a subject having a homozygous mutant of CCR8 may be HIV resistant or exhibit a slower rate of disease progression. Along the same lines, a subject having a heterozygous mutation in CCR8 may exhibit a slower rate of disease progression than a patient having a wild type CCR8. Mutations included in the CCR8 coding region may also result in inactivating mutations. In addition, a mutation in the regulatory region of CCR8 gene may prevent or inhibit expression of CCR8, thereby providing resistance to some degree from HIV infection.

Once an individual having a homozygous or heterozygous mutant in CCR8 is identified, it is envisioned that cells from that individual, once matched for histocompatibility, can be transplanted to an HIV positive individual, or to an "at risk" individual.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following

examples are to be considered illustrative and thus are not limiting of the remainder of the disclosure in any way whatsoever.

Example 1

Materials and Methods

Genomic Cloning and Sequencing Genomic DNA from a healthy donor was amplified by PCR using degenerate primers designed from conserved sequences in the predicted 3rd and 7th transmembrane domains of CXCR2 (Genbank #M73969) and an orphan receptor named 9-6 (Genbank # U45982). The primer sequences included *Hinc* II sites for cloning purposes, and are CC GTC GAC TGC ATI (T/A)(C/G)I GTI GA(C/T) (C/A)GI TA (primer CY3; SEQ ID NO:3), and CC GTC GAC AI IGG (A/G)TT IA(A/G) (G/A)CA I(G/C)(A/T) (A/G)TG (primer CY7; SEQ ID NO:4). The reaction contained 1.3 µg template DNA, 1 µM of each primer, 200 µM dATP, dTTP, dCTP and dGTP, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$ and 2.5 units of DNA polymerase (Perkin-Elmer Cetus) in 100 µl, and was amplified for 33 cycles (93°C for 1.5 min, 50°C for 2 min, and 72°C for 2 min), then given a final 7 min extension at 72°C. Products were cloned into the *Hinc* II site of pUC18 and sequenced (Song *et al.*, *Molecular cloning of a novel candidate G protein-coupled receptor from rat brain*, FEBS Lett. 351:375-379 (1994)). A novel sequence named CY6 was identified and used as a probe to isolate a human genomic clone from a lambda library (Lawn *et al.*, *The isolation and characterization of linked delta- and beta-globin genes from a cloned library of human DNA*, Cell 15:1157-1174 (1978)). A 1.9 kb fragment containing the CY6 ORF was isolated by cutting an *Eco* RI site in the vector and a genomic *Xba* I site, subcloned into pUC18 and sequenced completely on both strands. The 5' end of CY6 RNA was obtained using Clontech Marathon-Ready human thymus cDNA and nested primers from the coding region, named CY6A (CCAGAAGACTGAATACAAACAGGAGGCAA) SEQ ID NO:5 and CY6B (GTCTGAATAAGTTCCGCATCACAGGGGCTT). SEQ ID NO:6 The cDNA template was amplified using 10 pmol of CY6A and adaptor primer AP1 (30 cycles of 90°C 1 min, 60°C 1min, 72°C 2 min). Product from this reaction was

reamplified using CY6B and AP2 primers. The 200-250 bp product was gel-purified, digested with *Not* I and *Eco* RV (which cuts immediately 5' of the CY6B primer), cloned into Bluescript and sequenced.

Mapping. Fluorescence in situ hybridization (FISH) was carried out as previously described using the CY6 genomic clone as probe (Tory *et al.*, *A genetic linkage map of 96 loci on the short arm of human chromosome 3*, Genomics 13:275-286 (1992)). Radiation hybrid mapping was performed by PCR using the Stanford G3 panel (Research Genetics) with primers CY6B and CY6 (GCTAGGATTACAGGCATGAGCCACA) SEQ ID NO:7 to give a 341 bp product.

Creation of Cell Lines Expressing Chemokine receptors. The CY6 ORF was first amplified from the 1.9 kb genomic fragment using primers 5'-GCTCTAGATCTGTGACCAGGTCCCGCTGCC SEQ ID NO:8 (upper strand), which contains an *Xba* I site (underlined) and nucleotides -4 to -25 relative to the ATG initiator, and 5'-CGGAATTCATATTTAGTCTTCATTGATCCT SEQ ID NO:9 (lower strand), which contains an *Xho* I site (underlined) and 21 nucleotides downstream of the stop codon. The PCR product was subcloned into pcDNA3 (Invitrogen). Using the same methodology, we created Flag epitope-tagged constructs in pcDNA3 for CCR1, CCR3 and CCR5 using the p4 (GenBank #L10918), clone 3 (Genbank #U28694), and 8.5 (GenBank #U57840) cDNAs, respectively, as templates. The nucleotide sequences were confirmed on both strands. 4DE4 pre-B cell lymphoma cells (gift of L. Staudt, NCI) were grown in RPMI 1640 containing 10% FCS and 50 μM 2-mercaptoethanol. Human embryonic kidney (HEK) 293 cells were grown in DMEM with 10% FCS containing streptomycin 100 μg/ml and penicillin 100 U/ml. 1-1.5 X 10^7 cells in log phase were electroporated using a GenePulser (Bio-Rad Laboratories, Hercules, CA) with 20 μg of plasmid DNA. HEK 293 cell colonies resistant to 2 g/L G-418 (Gibco BRL) were isolated and expanded in the same media supplemented with 2 g/L G-418. 4DE4 cells were cultured in 1 g/L G-418 and expanded. For CCR1 and CCR3, mixed populations of 4DE4 cells resistant to G-418 were enriched for receptor-expressing cells by

chemotaxis in response to appropriate agonists through a ChemoTx chemotactic chamber (Neuroprobe Inc., Cabin John, MD) with a 5 μm pore size. Clones were obtained by limiting dilution, and receptor expression confirmed by FACS using the anti-Flag mAb Bio M5 according to the manufacturer's instructions (Kodak, Rochester, NY).

*Cell Culture*The promyelocytic cell line HL-60 clone 15 (CRL 1964, American Type Culture Collection, Rockville, MD) was maintained and induced to differentiate to eosinophil-like cells by treatment with 0.5 μM butyric acid (Sigma) and 10 ng/ml IL-5 (R&D, Minneapolis, MN), as previously described (Fischkoff *et al.*, *Graded increase in probability of eosinophilic differentiation of HL-60 promyelocytic leukemia cells induced by culture under alkaline conditions*, Leuk. Res. 12:679-686 (1988); Tiffany *et al.*, *Hyperglycosylation of eosinophil ribonucleases in a promyelocytic leukemia cell line and in differentiated peripheral blood progenitor cells*, J. Leukocyte Biol. 58:49-54 (1995)). Human neutrophils and mononuclear cells were purified from the peripheral blood of healthy donors. Mononuclear cells were plated on tissue culture plastic for 18 h and the adherent and non-adherent cells, enriched in monocytes and lymphocytes respectively, were collected separately for RNA analysis.

RNA Analysis. Total RNA was prepared using a commercial kit (Stratagene, La Jolla, CA). Blots were prepared and hybridized with ^{32}P-labeled probes as previously described (Tiffany *et al.*, *supra*).

Intracellular [Ca^{2+}] Measurements. Cells (10^7/ml) were incubated in PBS pH 7.4 and 2.5 μM FURA-2 AM (Molecular Probes, Eugene, OR) for 30-60 min at 37°C in the dark. The cells were subsequently washed twice with HBSS, and resuspended at $1X10^6$ cells/ml. One million cells were stimulated in a total volume of 2 ml in a continuously-stirred cuvette at 37°C in a fluorimeter (Photon Technology Inc., South Brunswick, NJ). Recombinant human chemokine sources: SDF-1β, HCC-1 and I-309, R&D; NAP-2, Bachem (Philadelphia, PA); the BB10010 variant of MIP-1α, a generous gift of L. Czaplewski (British Biotech, Inc.); all others, Peprotech (Rocky Hill, NJ). C3a was a

gift of C. Hammer. fMLP and recombinant human C5a were from Sigma (St. Louis, MO). The data were recorded every 200 msec as the relative ratio of fluorescence emitted at 510 nm after sequential excitation at 340 and 380 nm. For some experiments, cells were incubated with 250 ng/ml pertussis toxin, 2 μg/ml cholera toxin, or 2 μM herbimycin A for 4 h prior to functional assay

Chemotaxis. Cells were harvested and washed twice with PBS, then resuspended in serum-free RPMI 1640. Cells were loaded in a total volume of 25 μl into the upper compartment of a microchemotaxis chamber (Neuroprobe, Cabin John, MD). Chemoattractants were loaded in a final volume of 31 μl at indicated concentrations in the lower compartment. The two compartments were separated by a polyvinylpyrollidone-free polycarbonate filter with 5 μm pores. The chemotaxis chamber was incubated at 37°C, 100% humidity and 5% CO_2 for 4 h. The filter was then removed, and the number of cells migrating into each bottom compartment were counted using a hemocytometer. All conditions were tested in triplicate.

Example 2

Cloning of the Gene for CCR8

Taking advantage of the observation that G protein-coupled receptor genes have conserved sequences and often lack introns in the coding region, degenerate PCR primers were used to amplify a novel human genomic sequence named CY6 related to CC chemokine receptors. A 1953 bp fragment of a genomic clone containing the CY6 sequence was then isolated and sequenced. It extended to the 5'-end of the phage insert, and contained a 1065 bp ORF, and 250 and 620 bp of 5' and 3' sequence, respectively. The deduced protein sequence is most closely related to CCR1-5 (39-43% identity) with lower identity (25-30%) to CXC chemokine receptors. To confirm the initiation codon, we first identified thymus as a rich natural source of CY6 mRNA and used it to amplify the 5'-UTR sequence by anchored PCR. The sequence revealed 120 bases 5' of the putative ATG initiator (GenBank # pending) with an in frame terminator 15 bases 5' of

the ATG and residing at the 3' end of an upstream exon, strongly supporting this codon as the initiator.

The CY6 genomic fragment was mapped to human chromosome 3p22-p23 by FISH. Forty nine cells were examined, with 25 showing paired hybridization signal and 17 showing a single signal at 3p23-p22. Two point linkage analysis of the radiation hybrid data by the Stanford Radiation Hybrid server gives a LOD of 11.5 for linkage to D3S3527 at a distance of 5.4 cR_{10000}. This places the gene between the Genethon markers D3S1260 and D3S3522, and between the gene for CCR4 and a cluster of genes for CCR1, 2, 3 and 5 (Samson *et al.*, *The genes encoding the human CC-chemokine receptors CC-CKR1 to CC-CKR5 (CMKBR1-CMKBR5) are clustered in the p21.3-p24 region of chromosome 3*, Genomics 36:522-526 (1996)). Using FISH, Napolitano et al reported that TER1 maps to chromosome 3p21 (Napolitano *et al.*, *Molecular cloning of TER1, a chemokine receptor-like gene expressed by lymphoid tissues*, J. Immunol. 157:2759-2763 (1996)).

Using a multi-tissue Northern blot, mRNA was detected at high levels in thymus, and at lower levels in spleen, but not in 14 other tissues tested. Using Northern blots from the same supplier, the same RNA distribution pattern has been reported by Napolitano et al and Zaballos et al (Napolitano *et al.*, *Molecular cloning of TER1, a chemokine receptor-like gene expressed by lymphoid tissues*, J. Immunol. 157:2759-2763 (1996); Zaballos *et al.*, *Molecular cloning and RNA expression of two new human chemokine receptor-like genes*, Biochem. Biophys. Res. Commun. 227:846-853 (1996)). Napolitano et al also detected transcripts in the MOLT-4 T cell line and the NK3.3 NK cell line, but not in primary NK cells, monocytes, neutrophils or PHA/PMA-activated PBMCs (Napolitano *et al.*, *supra*). A 4.6 kb mRNA band was detected in total RNA from adherent monocytes, consistent with the size in thymus, but not in neutrophil or lymphocyte samples (Fig. 1). Zaballos et al also detected mRNA in monocyte/macrophages, as well as in lymph node, and CD4+, CD8+ and CD19+ lymphocytes (Zaballos *et al.*, *supra*).

Because of the functional specificity that we now describe, we have provisionally named the protein product of the CY6/TER1 ORF CC chemokine receptor 6 or CCR8. This is in keeping with a nomenclature system accepted by consensus at the 2nd Gordon Conference on Chemotactic Cytokines (Plymouth, NH, 1997).

Agonists for CCR8. To identify a specific agonist, we screened a panel of chemoattractants for the ability to induce calcium flux in the mouse pre-B cell line 4DE4 before and after transfection with a plasmid encoding CCR8. Untransfected 4DE4 cells did not respond to any agonists tested except for the CXC chemokine SDF-1 (Fig. 2A). 4DE4 cells transfected with the CCR8 plasmid exhibited $[Ca^{2+}]_i$ transients in response to SDF-1 and I-309, but not in response to the following tested at 50 nM or greater: the CC chemokines HCC-1, MIP-1α, RANTES, MIP-1β, MCP-1, MCP-2, MCP-3, MCP-4 and eotaxin; the CXC chemokines IL-8, γIP-10, NAP-2, GROα, GROβ, GROγ and ENA-78; the C chemokine lymphotactin; and the non-chemokine leukocyte chemoattractants fMLP, C3a and C5a (not shown). I-309 did not induce calcium flux in 4DE4 cell lines expressing CCR1 or CCR3, or in HEK 293 cells stably expressing CCR5 (Fig. 2A, and data not shown). The CCR1, CCR3 and CCR5 cell lines all responded appropriately to previously described agonists (Fig. 2A, and data not shown).

The threshold for the calcium flux response of CCR8-expressing cells to I-309 was 0.1 nM, and the EC_{50} was 2 nM (Fig. 2B). These values are similar to those observed for other chemokine receptors (7-14). When the cells were pre-treated with other ineffective chemokines or with SDF-1, there was no effect on the magnitude or kinetics of the I-309-induced calcium flux response, suggesting that other chemokines are not antagonists at CCR8. In contrast, when cells were sequentially stimulated with the same concentration of I-309, no response to the second application was observed (Fig. 2A), suggesting homologous desensitization of the receptor.

I-309 was able to induce transmigration of 4DE4 cells expressing CCR8, but not untransfected cells, across a filter in a modified Boyden chamber assay of chemotaxis

(Fig. 3). The I-309 dose-response curve for chemotaxis was bell-shaped, which is typical for this response. I-309 was both highly potent (EC_{50} of 2 nM and an optimal concentration of 5 nM) and highly efficacious (~40% of input cells migrated across the filter at the optimal concentration). Checkerboard analysis indicated that the response was chemotactic and not chemokinetic.

Example 3

Development of an HL-60 Cell Line Model for Endogenous CCR8

The clone 15 variant of HL-60 cells can be induced by butyric acid and IL-5 treatment to differentiate within 2 days to cells having many of the characteristics of peripheral blood eosinophils, including expression of eosinophil-specific granule proteins (Fischkoff *et al.*, *supra*; Tiffany *et al.*, *supra*). Using Northern blot analysis, we were unable to detect mRNA for CCR8 in the uninduced cells, and the cells did not respond to I-309 in either the calcium flux or chemotaxis assays (Fig. 4A-C). However, when the cells were cultured in the presence of butyric acid and IL-5, a 4.6 kb band was detected by Northern blot using a CCR8 ORF probe (Fig. 4A).

Induction of CCR8 mRNA correlated with acquisition of calcium flux responses to I-309 (Fig. 4B). The EC_{50} was 1 nM (Fig. 4C), similar to the value observed for I-309-induced calcium flux and chemotaxis in 4DE4 cells expressing recombinant CCR8. Induced cells also responded to MIP-1α, RANTES, MCP-3 and eotaxin, consistent with the induction of CCR1 and CCR3 mRNA (HL Tiffany and PM Murphy, unpublished observations), but none of these chemokines was able to desensitize I-309-induced calcium flux, consistent with their lack of agonist and antagonist activity at CCR8 (Fig. 4D). I-309-induced calcium flux in both differentiated HL-60 clone 15 cells and 4DE4 cells expressing CCR8 was abolished by treatment with pertussis toxin, but not by cholera toxin or herbimycin A, suggesting specific coupling of the receptor to G proteins of the G_i class in both cell types (Fig. 5). Although the HL-60 clone 15 cells are a useful model system for studying endogenous CCR8, it is important to point out that we have not been

able to demonstrate CCR8 mRNA or I-309 responsiveness in primary human eosinophils, even when stimulated with IL-5.

Identification of CCR8 is an essential first step in understanding the mechanism of action of I-309. The ability of recombinant CCR8 to support chemotaxis in transfected pre-B cells suggests that endogenous CCR8 may mediate I-309's chemotactic activity in monocytes. The pattern of constitutive CCR8 mRNA expression in tissues that we and others (Napolitano *et al.*, *supra*; Zaballos *et al.*, *supra*) have observed is unique relative to known chemokine receptors, and suggests a role for CCR8 specifically in thymus. This is consistent with the ability of I-309 to inhibit dexamethasone-induced apoptosis in mouse thymic cell lines (Van Snick *et al.*, *I-309/T cell activation gene-3 chemokine protects murine T cell lymphomas against dexamethasone-induced apoptosis*, J. Immunol. 157:2570-2576 (1996)). Together, these observations suggest the importance of future studies to test the role of I-309 in thymocyte migration and development in vivo. This may be accomplished by targeted genetic disruption of mouse CCR8, which has not yet been identified, or of TCA3, which appears to be the mouse homologue of I-309. Like I-309, TCA3 induces monocyte chemotaxis. It has also been shown to induce degranulation, production of reactive nitrogen intermediates, and upregulation of adhesion molecules in monocytes, but, unlike I-309, has been reported to have parallel activities on neutrophils. TCA3 can also suppress the growth of certain tumors in both immunocompetent and immunodeficient mice (Luo *et al.*, *Biologic activities of the murine beta-chemokine TCA3*, J. Immunol. 153:4616-4624 (1994); Devi *et al.*, *Biologic activities of the beta-chemokine TCA3 on neutrophils and macrophages*, J. Immuno. 154:5376-5383 (1995); Laning et al., *Inhibition of in vivo tumor growth by the beta chemokine, TCA3*, J. Immunol. 153:4625-4635 (1994)).

Like other chemokines, I-309 is likely to provide directional information for orderly leukocyte trafficking in vivo (Springer *et al.*, *Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm*, Cell 76:301-314 (1994)). Like other chemokines, if it is dysregulated, I-309 has the potential to cause inappropriate

inflammation and tissue injury. In this regard, our identification of an I-309 receptor may be useful in future research aimed at evaluating this pathway for development of potential anti-inflammatory therapies.

It will be apparent to those skilled in the art that various modifications and variations can be made to the compositions and processes of this invention. Thus, it is intended that the present invention cover such modifications and variations, provided they come within the scope of the appended claims and their equivalents.

What Is Claimed Is:

1. A recombinant cell line that expresses CCR8 polypeptide.

2. The cell line of claim 1, wherein the cells coexpress human CD4 and CCR8.

3. A recombinant host cell stably transformed with a polynucleotide encoding CCR8 polypeptide, wherein the cell co-expresses CCR8 and CD4 polypeptide.

4. A recombinant host cell stably transformed with a polynucleotide encoding CCR8 polypeptide and a polynucleotide encoding CD4 polypeptide, wherein the cell co-expresses CCR8 and CD4 polypeptide.

5. The cell as in any of claims 1-4, wherein the cell is a human cell.

6. The cell as in any of claims 1-4, wherein the cell is a non-human cell.

7. An antibody which specifically binds to CCR8 polypeptide or fragments thereof.

8. The antibody of claim 7, wherein the antibody blocks membrane fusion between HIV and a target cell.

9. The antibody of claim 7, wherein the antibody is a monoclonal antibody.

10. The antibody of claim 7, wherein the antibody blocks chemokine binding.

11. A cell that expresses a CCR8 gene, wherein the CCR8 gene is not stably integrated into the genome of the cell.

12. The cell of claim 11, wherein the cell also expresses a CD4 gene.

13. An isolated polynucleotide which encodes an amino acid sequence as set forth in SEQ ID NO:1.

14. An isolated polynucleotide selected from the group consisting of:

a) SEQ ID NO:1;

b) SEQ ID NO:1,wherein T can also be U;

c) nucleic sequences complementary to SEQ ID NO:1; and

d) fragments of a), c), or e) that are at least 15 bases in length and that will selectively hybridize to genomic DNA which encodes the CCR8 protein of SEQ ID NO:2.

15. An expression vector containing in operable linkage the polynucleotide as in claim 14.

16. A host cell containing the vector of claim 15.

17. A substantially purified peptide fragment of CCR8, wherein the peptide inhibits cell membrane fusion between HIV and a target cell or between an HIV-infected cell and a CD4 positive uninfected cell.

18. A substantially purified CCR8-binding agent, wherein the binding agent inhibits membrane fusion between HIV and a target cell or between an HIV-infected cell and a CD4 positive uninfected cell.

19. The agent of claim 18, wherein the agent is selected from a biologic agent and a chemical compound.

20. The agent of claim 18, wherein the biologic agent is a chemokine.

21. The agent as in claims 18 or 20, wherein the agent blocks chemokine binding to CCR8.

22. A method of inhibiting membrane fusion between HIV and a target cell or between an HIV-infected cell and a CD4 positive uninfected cell comprising contacting the target or CD4 positive cell with a fusion-inhibiting effective amount of a CCR8 binding or blocking agent.

23. The method of claim 22, wherein the agent is a anti-CCR8 antibody or epitope binding fragment thereof.

24. The method of claim 23, wherein the antibody is a monoclonal antibody or a polyclonal antibody.

25. The method of claim 24, wherein the contacting is by *in vivo* administration to a subject.

26. The method of claim 25, wherein the anti-CCR8 antibody is administered by intravenous, intra-muscular or subcutaneous injections.

27. The method of claim 26, wherein the anti-CCR8 antibody is administered within a dose range of 0.1 ug/kg to 100 mg/kg.

28. The method of claim 27, wherein the antibody is formulated in a pharmaceutically acceptable carrier.

29. A method for identifying a compound which binds to CCR8 polypeptide comprising:

a) incubating components comprising the compound and CCR8 polypeptide under conditions sufficient to allow the components to interact; and

b) measuring the binding of the compound to CCR8 polypeptide

30. The method of claim 29, wherein the compound is a peptide.

31. The method of claim 29, wherein the compound is a peptidomimetic.

32. The method of claim 29, wherein the CCR8 polypeptide is expressed in a cell.

33. The method of claim 32, wherein the cell is the cell of claim 1.

34. A method for identifying a compound which blocks membrane fusion between HIV and a target cell or between an HIV-infected cell and a CCR8 positive uninfected cell comprising:

a) incubating components comprising the compound and a CCR8 positive cell under conditions sufficient to allow the components to interact;

b) contacting the components of step a) with HIV or an HIV-infected cell; and

c) measuring the ability of the compound to block membrane fusion between HIV and the CCR8 positive cell or between an HIV-infected cell and a CCR8 positive uninfected cell.

35. The method of claim 34, wherein the CCR8 postive cell is a CD4 positive cell.

36. The method of claim 34, wherein measuring the ability of the composition to block membrane fusion is by detection of a reporter means.

37. The method of claim 36, wherein the reporter means is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator, or an enzyme.

38. The method of claim 37, wherein the reporter means is a *lacZ* gene.

39. A transgenic non-human animal having a phenotype characterized by expression of CCR8 polypeptide and CD4 polypeptide otherwise not naturally occurring in the animal, the phenotype being conferred by a transgene contained in the somatic and germ cells of the animal, the transgene comprising a nucleic acid sequence which encodes CCR8 polypeptide and a nucleic acid sequence which encodes CD4 polypeptide.

40. The transgenic non-human animal of claim 39, wherein the animal is a mouse.

41. The transgenic non-human animal of claim 39, wherein the animal is a rabbit.

42. A transgenic non-human animal having a phenotype characterized by expression of CCR8 polypeptide otherwise not naturally occurring in the animal, the phenotype being conferred by a transgene contained in the somatic and germ cells of the animal, the transgene comprising a nucleic acid sequence which encodes CCR8 polypeptide.

43. A method for producing a transgenic non-human animal having a phenotype characterized by expression of CCR8 polypeptide and CD4 polypeptide otherwise not naturally occurring in the animal, the method comprising:

(a) introducing at least one transgene into a zygote of an animal, the transgene(s) comprising a DNA construct encoding CCR8,

(b) transplanting the zygote into a pseudopregnant animal,

(c) allowing the zygote to develop to term, and

(d) identifying at least one transgenic offspring containing the transgene.

44. The method of claim 43, further comprising a DNA construct encoding CD4.

45. The method of claim 43, wherein the introducing of the transgene into the embryo is by introducing an embryonic stem cell containing the transgene into the embryo.

46. The method of claim 43, wherein the introducing of the transgene into the embryo is by infecting the embryo with a retrovirus containing the transgene.

47. The method of claim 43, wherein the animal is selected from the group consisting of a mouse and a rabbit.

48. A transgenic non-human animal having a transgene disrupting or interfering with expression of CCR8 chromosomally integrated into the germ cells of the animal.

49. The transgenic animal of claim 48, wherein the animal is selected from the group consisting of a mouse and a rabbit.

50. The transgenic non-human animal of claim 48, wherein the transgene comprises CCR8 antisense polynucleotide.

51. A method of treating a subject having or at risk of having an HIV infection or disorder, comprising administering to the subject, a therapeutically effective amount of an anti-CCR8 antibody, wherein the antibody inhibits cell-cell fusion in cells infected with HIV.

52. The method of claim 51, wherein the antibody is a monoclonal antibody.

53. The method of claim 52, wherein the monoclonal antibody is a humanized monoclonal antibody.

54. The method of claim 51, wherein the monoclonal antibody is administered to a patient suffering from AIDS or ARC.

55. The method of claim 52, wherein the monoclonal antibody is administered within a dose range between about 0.1/kg to about 100 mg/kg.

56. The method of claim 51, wherein the monoclonal antibody is formulated in a pharmaceutically acceptable carrier.

57. A method of treating a subject having an HIV-related disorder associated with expression of CCR8 comprising administering to an HIV infected or susceptible cell of the subject, a agent that suppresses CCR8.

58. The method of claim 57, wherein the agent is an anti-CCR8 antibody.

59. The method of claim 57, wherein the agent is an antisense nucleic acid that hybridizes to a CCR8 nucleic acid.

60. The method of claim 57, wherein the agent is introduced into the cell using a carrier.

61. The method of claim 57, wherein the carrier is a vector.

62. The method of claim 57, wherein the administering is *ex vivo*.

63. The method of claim 57, wherein the administering is *in vivo*.

64. A pharmaceutical composition comprising CCR8 in a pharmaceutically acceptable carrier.

65. A method for inhibiting the expression of CCR8 in a cell comprising contacting CCR8 with an inhibiting effective amount of an antisense oligonucleotide that binds to a segment of an mRNA transcribed from a CCR8 gene, whereby the binding of the antisense to the mRNA segment inhibits CCR8 expression.

66. Substantially pure CCR8 polypeptide having an amino acid sequence as set forth in SEQ ID NO:2.

Fig.1

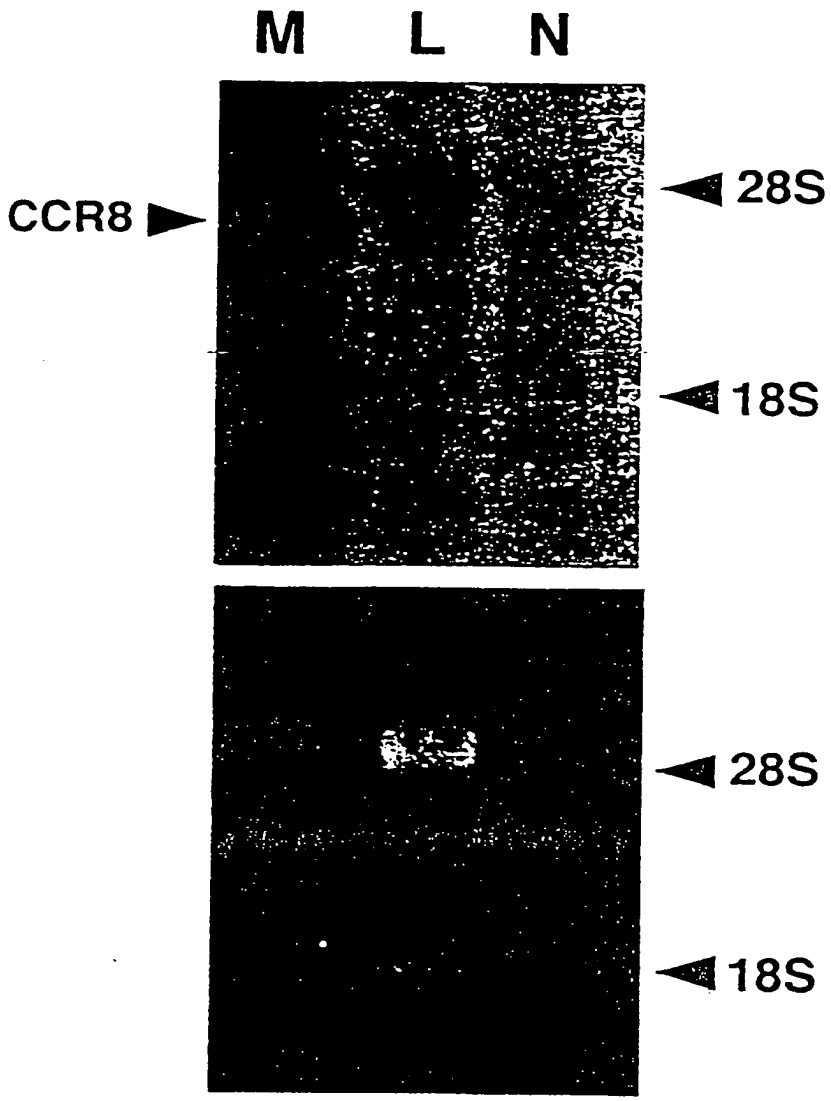


Fig.2

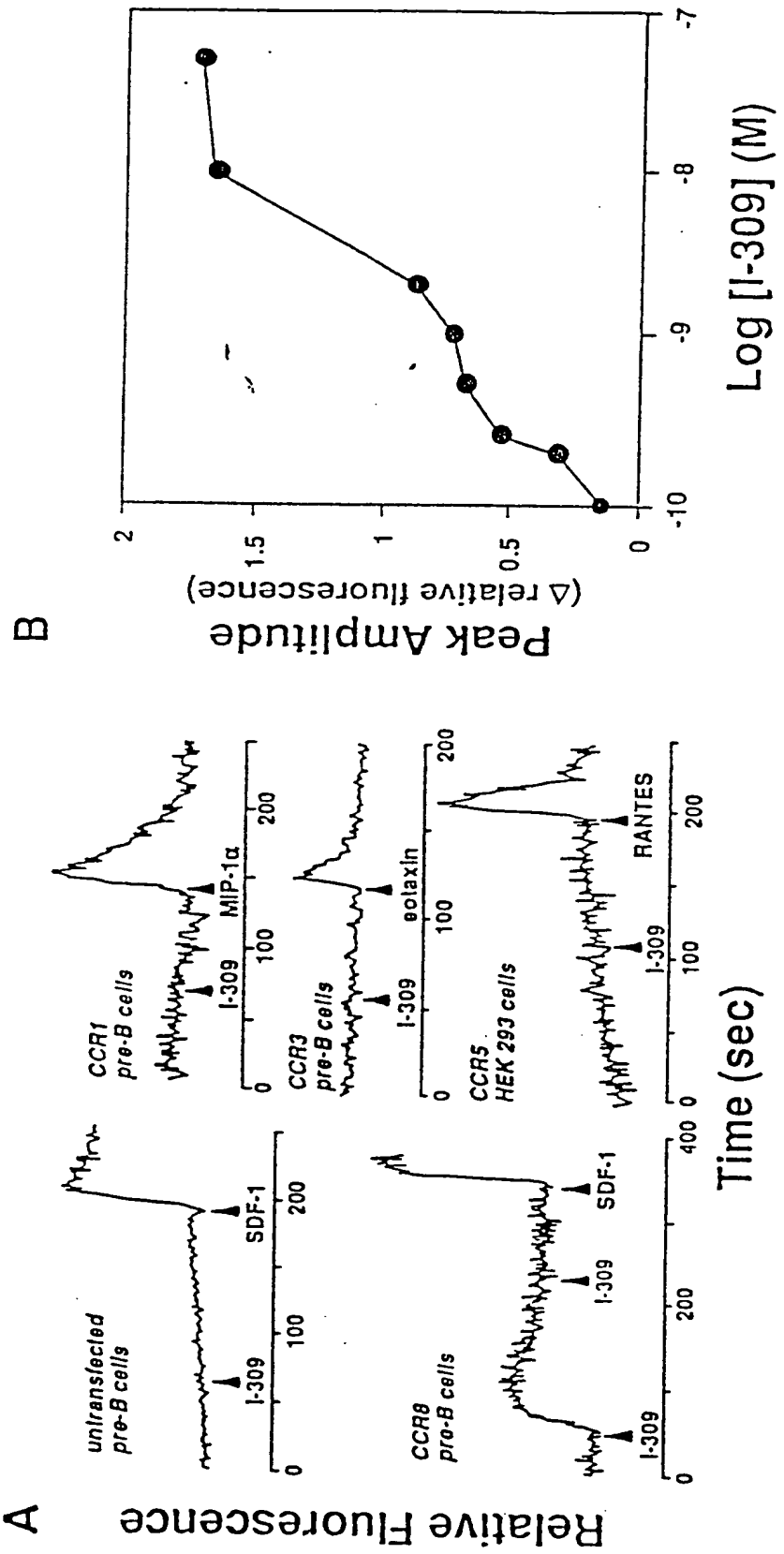


Fig .3

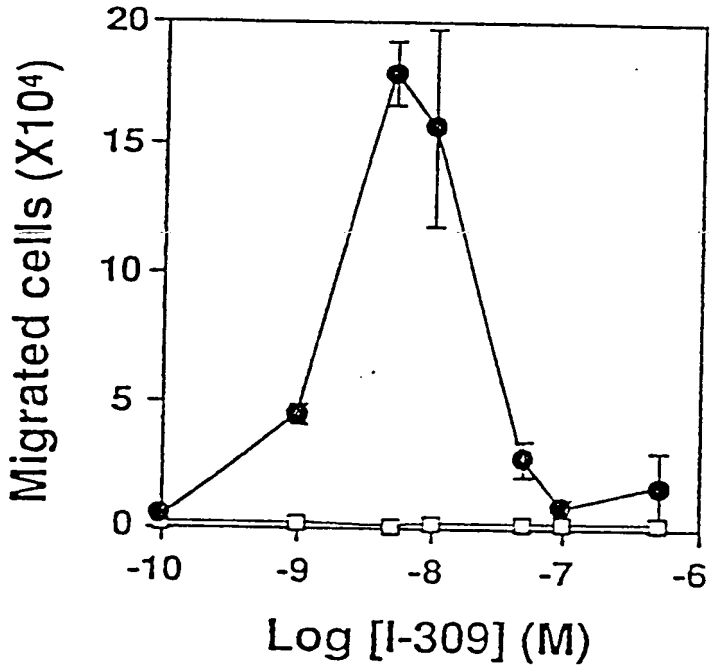


Fig.4

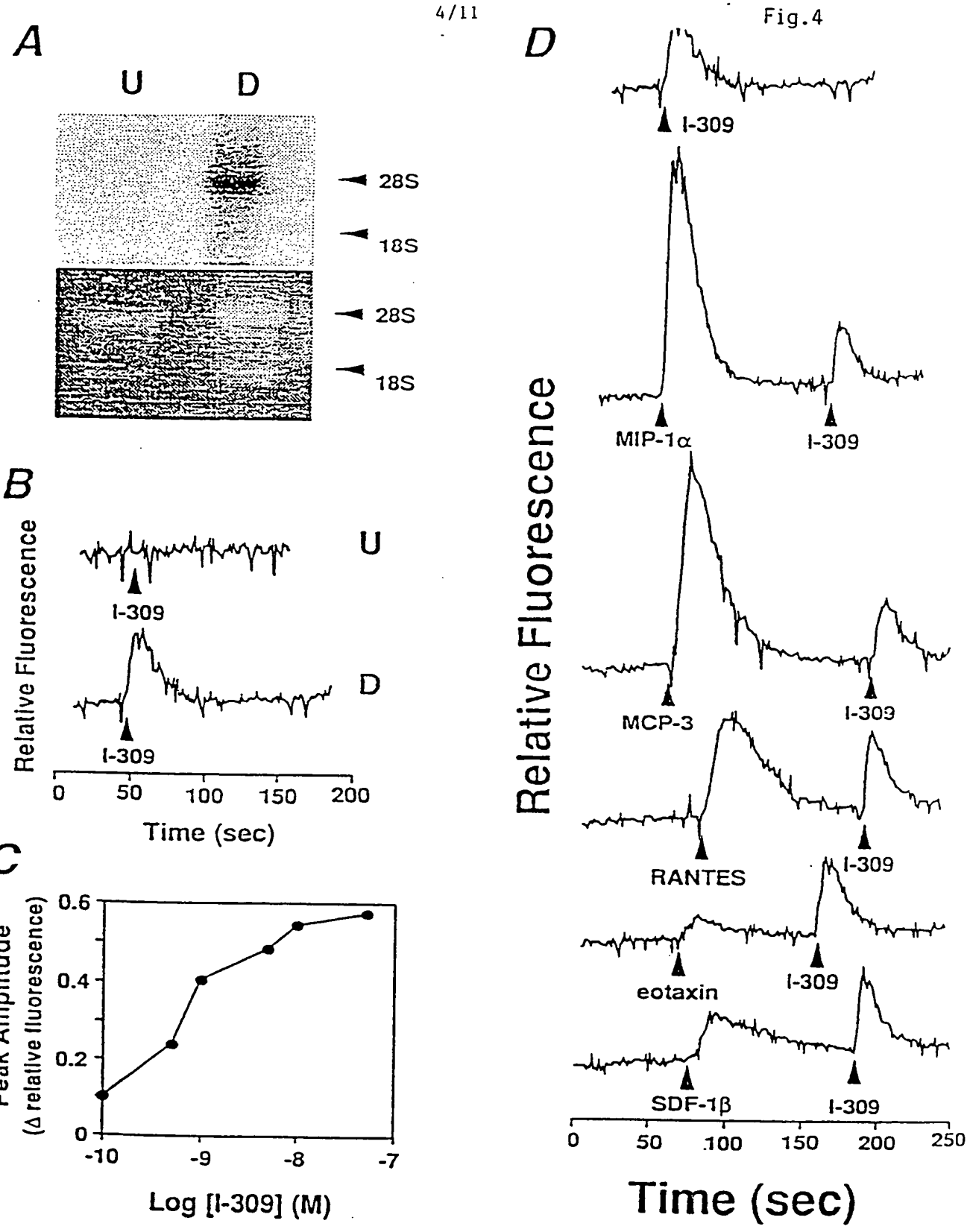


Fig.5

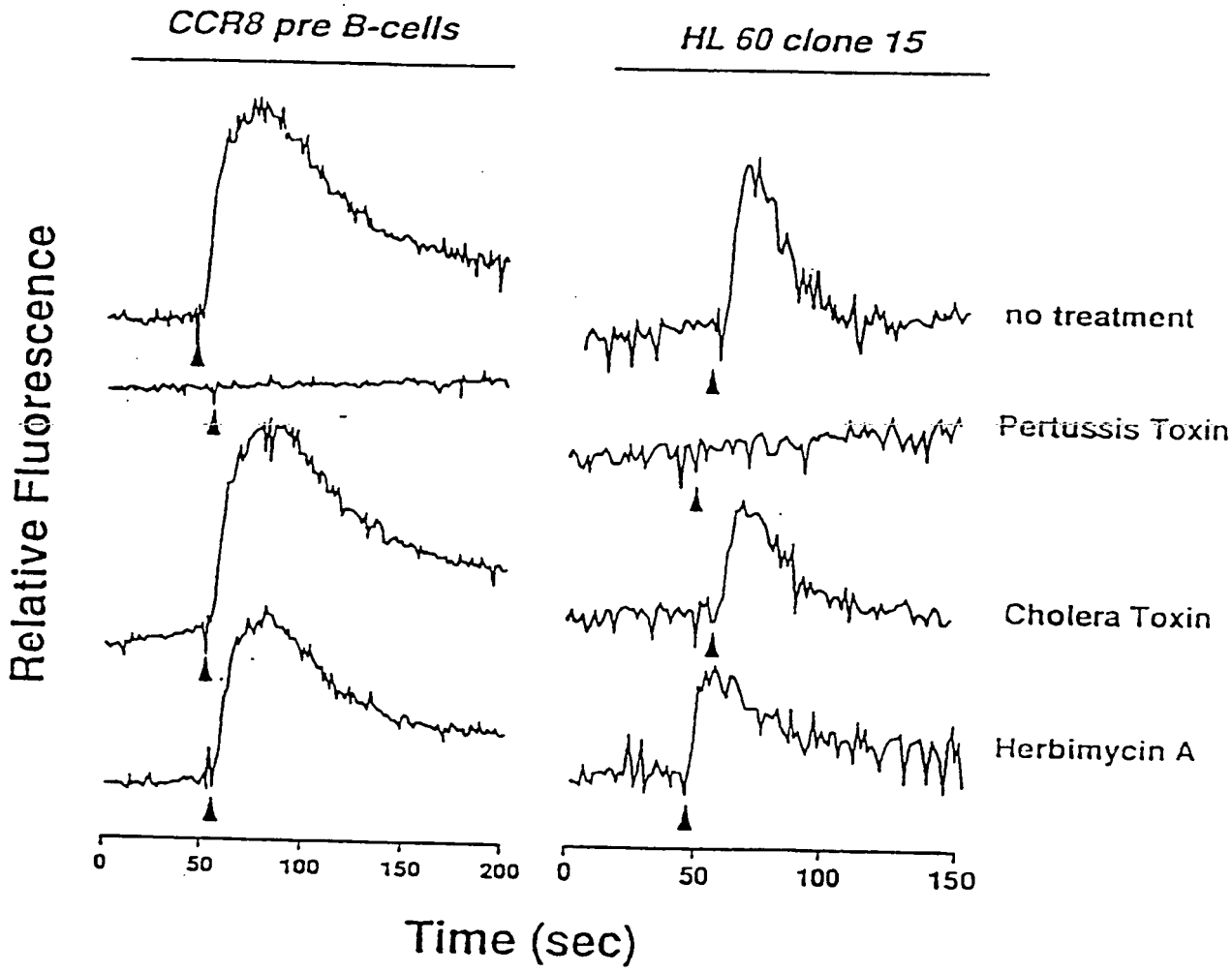

CCR8 pre B-cells
HL 60 clone 15
Relative Fluorescence
no treatment
Pertussis Toxin
Cholera Toxin
Herbimycin A
0
50
100
150
200
0
50
100
150
Time (sec)

Fig.6

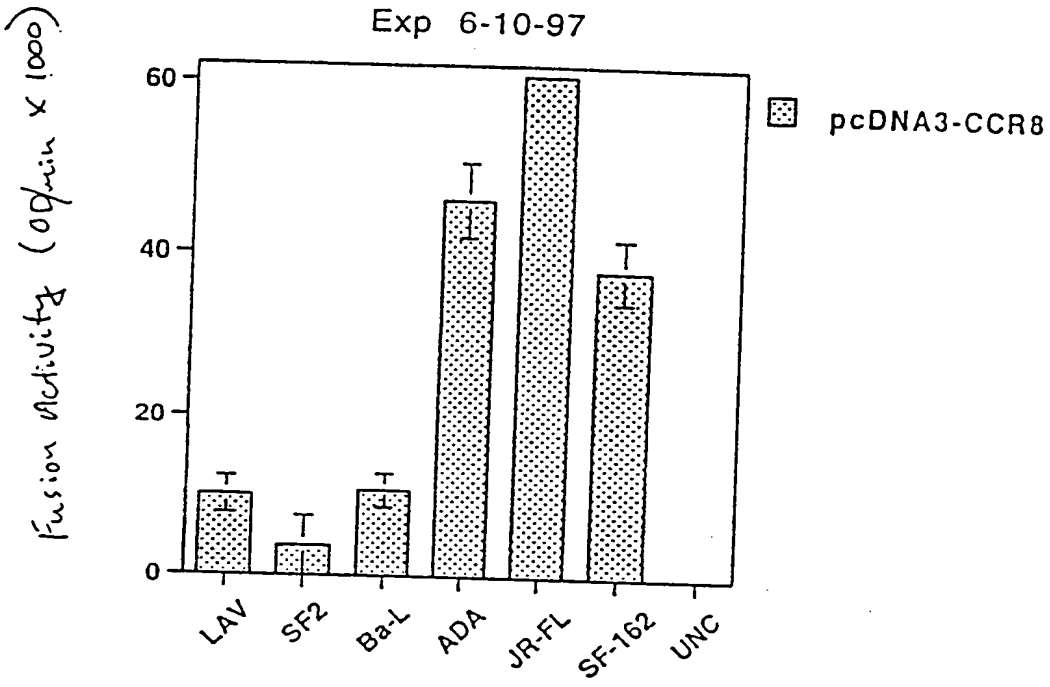

Exp 6-10-97
Fusion Activity (OD/min x 1000)
60
40
20
0
LAV
SF2
Ba-L
ADA
JR-FL
SF-162
UNC
pcDNA3-CCR8

Fig.7

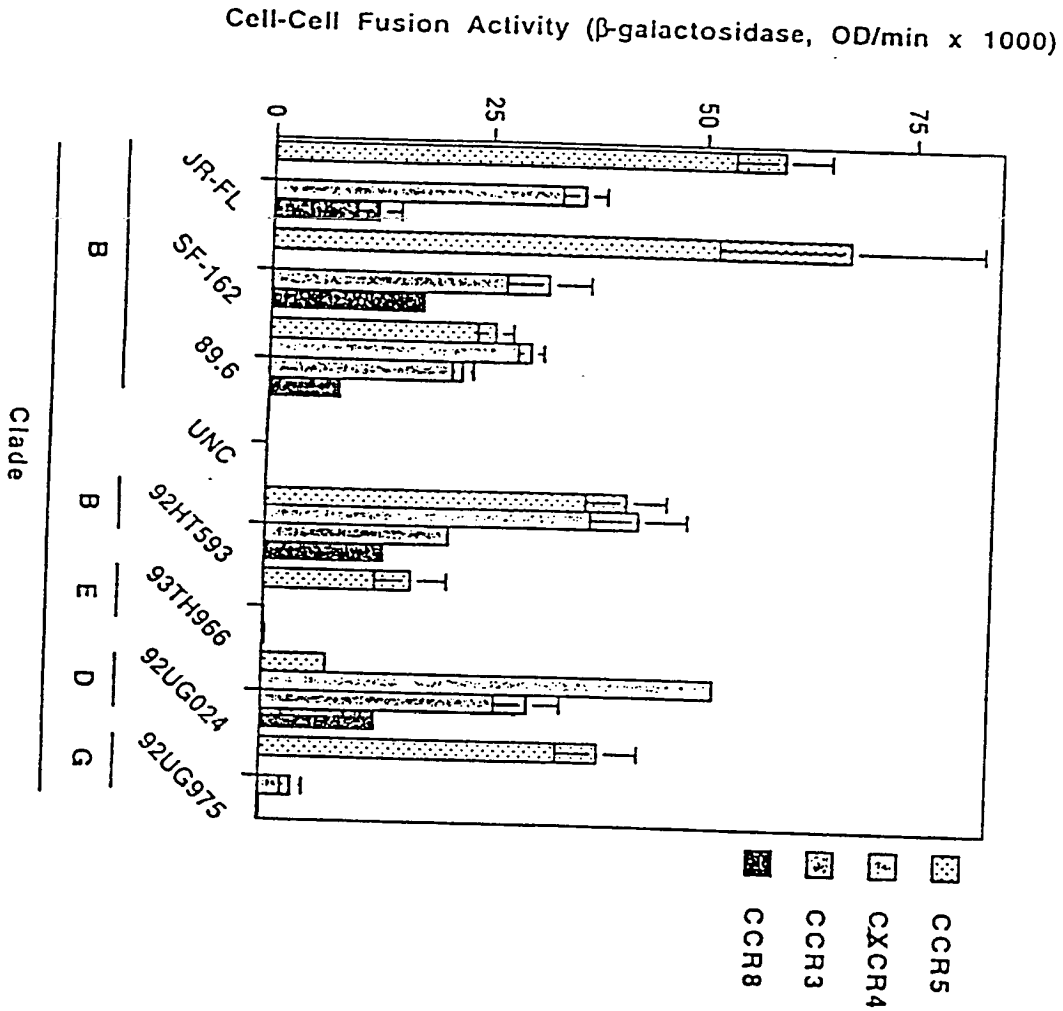

Cell-Cell Fusion Activity (β-galactosidase, OD/min x 1000)
0
25
50
75
Clade
B
JR-FL
SF-162
89.6
UNC
B
92HT593
E
93TH966
D
92UG024
G
92UG975
CCR8
CCR3
CXCR4
CCR5

Fig.8

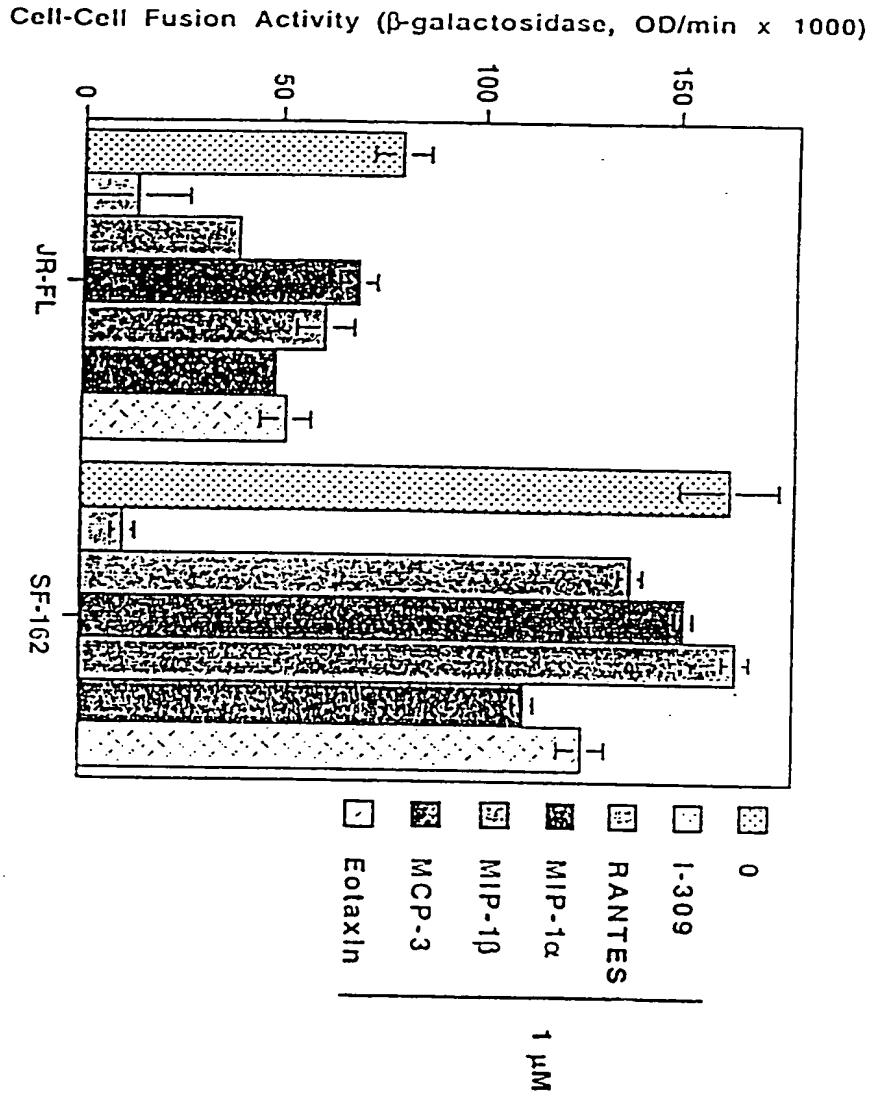
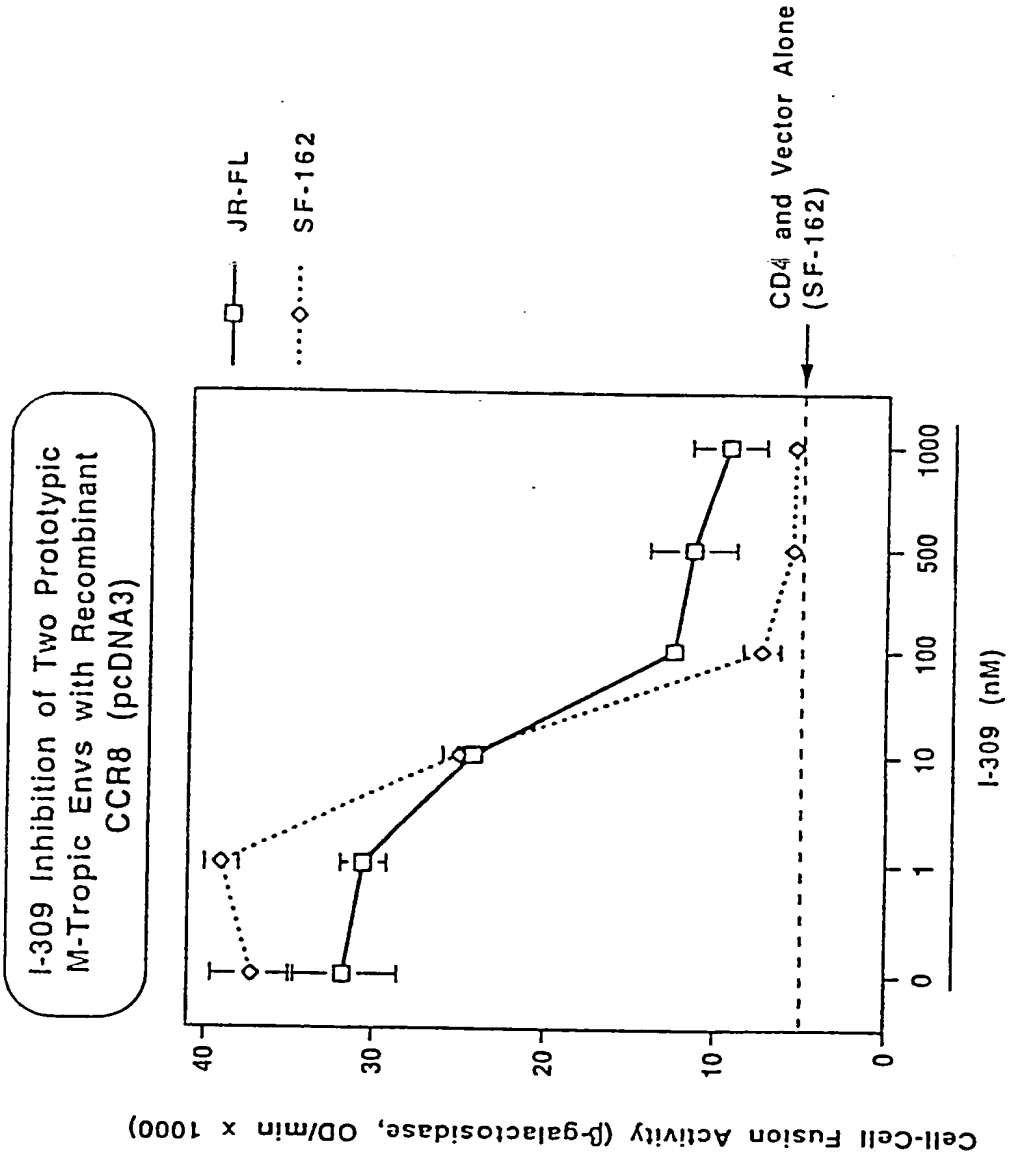

Cell-Cell Fusion Activity (β-galactosidase, OD/min x 1000)
0
50
100
150
JR-FL
SF-162
0
I-309
RANTES
MIP-1α
MIP-1β
MCP-3
Eotaxin
1 μM

Fig.9



```
1    CCTCCCAAAG TGCTAGGATT ACAGGCATGA GCCACAGCTC CCGGTCTATC

51   ATTTAACCTT AATTACATCT TTAAAGGCCC AAATAGTCTC ACCCACTCCA

101  AATAGTCACA CCCACACCGG AGGTTGAGCA CTTCAACACA TGAATTTGGG

151  GAGGACACAG TTCAGTCCAT AACATCCCCC TAATTTTTAA AAAATAAAAA

201  TGTTTTTAAG GAGTGAATGT CTTTTATGTG TCTCTGTGAC CAGGTCCCGC

251  TGCCTTGATG GATTATACAC TTGACCTCAG TGTGACAACA GTGACCGACT

301  ACTACTACCC TGATATCTTC TCAAGCCCCT GTGATGCGGA ACTTATTCAG

351  ACAAATGGCA AGTTGCTCCT TGCTGTCTTT TATTGCCTCC TGTTTGTATT

401  CAGTCTTCTG GGAAACAGCC TGGTCATCCT GGTCCTTGTG GTCTGCAAGA

451  AGCTGAGGAG CATCACAGAT GTATACCTCT TGAACCTGGC CCTGTCTGAC

501  CTGCTTTTTG TCTTCTCCTT CCCCTTTCAG ACCTACTATC TGCTGGACCA

551  GTGGGTGTTT GGGACTGTAA TGTGCAAAGT GGTGTCTGGC TTTTATTACA

601  TTGGCTTCTA CAGCAGCATG TTTTTCATCA CCCTCATGAG TGTGGACAGG

651  TACCTGGCTG TTGTCCATGC CGTGTATGCC CTAAAGGTGA GGACGATCAG

701  GATGGGCACA ACGCTGTGCC TGGCAGTATG GCTAACCGCC ATTATGGCTA

751  CCATCCCATT GCTAGTGTTT TACCAAGTGG CCTCTGAAGA TGGTGTTCTA

801  CAGTGTTATT CATTTTACAA TCAACAGACT TTGAAGTGGA AGATCTTCAC

851  CAACTTCAAA ATGAACATTT TAGGCTTGTT GATCCCATTC ACCATCTTTA

901  TGTTCTGCTA CATTAAAATC CTGCACCAGC TGAAGAGGTG TCAAAACCAC

951  AACAAGACCA AGGCCATCAG GTTGGTGCTC ATTGTGGTCA TTGCATCTTT

1001 ACTTTTCTGG GTCCCATTCA ACGTGGTTCT TTTCCTCACT TCCTTGCACA

1051 GTATGCACAT CTTGGATGGA TGTAGCATAA GCCAACAGCT GACTTATGCC

1101 ACCCATGTCA CAGAAATCAT TTCCTTTACT CACTGCTGTG TGAACCCTGT

1151 TATCTATGCT TTTGTTGGGG AGAAGTTCAA GAAACACCTC TCAGAAATAT

1201 TTCAGAAAAG TTGCAGCCAA ATCTTCAACT ACCTAGGAAG ACAAATGCCT

1251 AGGGAGAGCT GTGAAAAGTC ATCATCCTGC CAGCAGCACT CCTCCCGTTC
```

FIGURE 10A

```
1301  CTCCAGCGTA GACTACATTT TGTGAGGATC AATGAAGACT AAATATAAAA
1351  AACATTTTCT TGAATGGCAT GCTAGTAGCA GTGAGCAAAG GTGTGGGTGT
1401  GAAAGGTTTC CAAAAAAAGT TCAGCATGAA GGATGCCATA TATGTTGTTG
1451  CCAACACTTG GAACACAATG ACTAAAGACA TAGTTGTGCA TGCCTGGCAC
1501  AACATCAAGC CTGTGATTGT GTTTATTGAT GATGTTGAAC AAGTGGTAAC
1551  TTTAAAGGAT TCTGTATGCC AAGTGAAAAA AAAAGATGTC TGACCTCCTT
1601  ACATATGCAA AAATATACCT TCAGAGACTG TCAGTAGGCT GGAAGAAGTG
1651  GATATTGAAG TTTTGACATC AATGATGAGG CTCCAGTTGT CTATTCATTG
1701  ACTGATGGTG AAATGGCTGG AGTGATTCTG AATCAAGGTG ATTGTGATTA
1751  TAGTGACAAT GAAGATGATG CTATTAATAC TGCATAAAAA GTGCCTGTAG
1801  ATGACATGGT GAAAATATTT GACAGGCTTA TGGAAGGACT ACAGCAGCAC
1851  GCATTCATAA CAGAACAAGA AATTATGTCA GCTTATAAAA TCAAACAGAG
1901  ACTTCTAGAC AAAAACCATT GTTGATGAGG CAGATGCCTC TAGA
```

```
      MDYTLDLSVTTVTDYYYPDIFSSPCDAELIQTNGKLLLAVFYCL
LFVFSLLGNSLVILVLVVCKKLRSITDVYLLNLALSDLLFVFSFPFQTYYLLDQWVFG
TVMCKVVSGFYYIGFYSSMFFITLMSVDRYLAVVHAVYALKVRTIRMGTTLCLAVWLT
AIMATIPLLVFYQVASEDGVLQCYSFYNQQTLKWKIFTNFKMNILGLLIPFTIFMFCY
IKILHQLKRCQNHNKTKAIRLVLIVVIASLLFWVPFNVVLFLTSLHSMHILDGCSISQ
QLTYATHVTEIISFTHCCVNPVIYAFVGEKFKKHLSEIFQKSCSQIFNYLGRQMPRES
CEKSSSCQQHSSRSSSVDYIL*
```

FIGURE 10B

Fig. 1

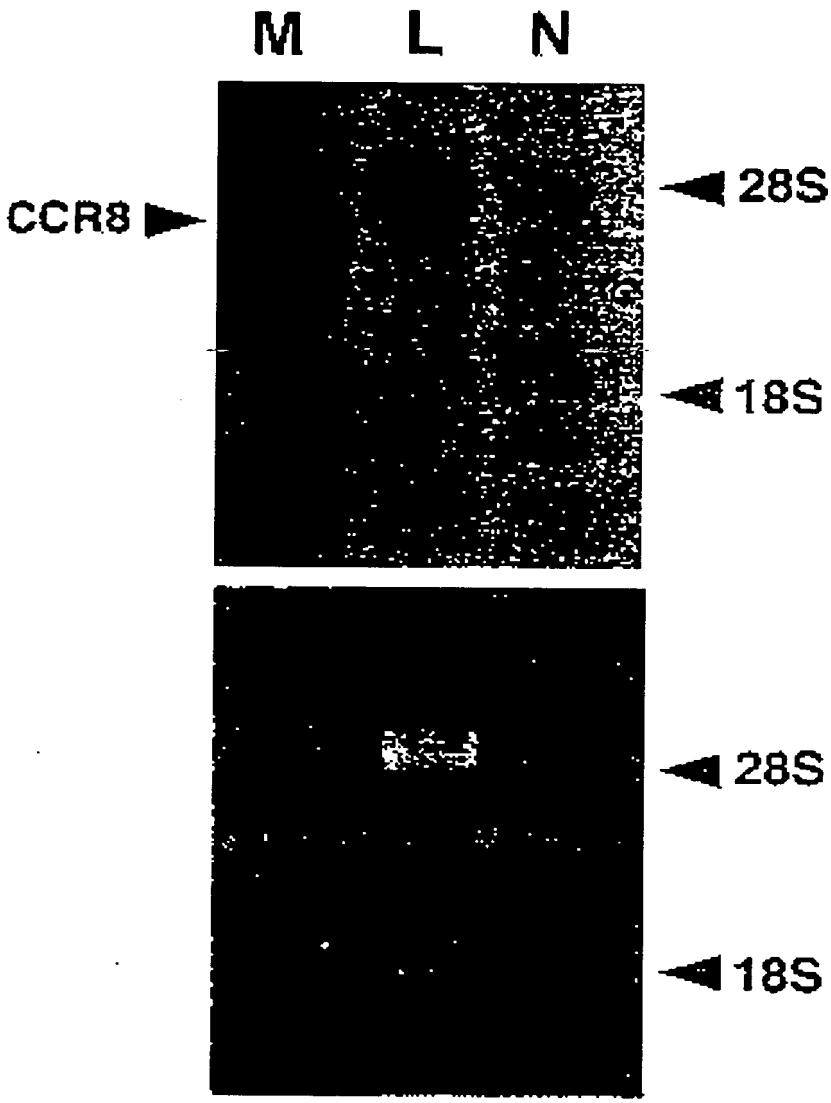


Fig.2

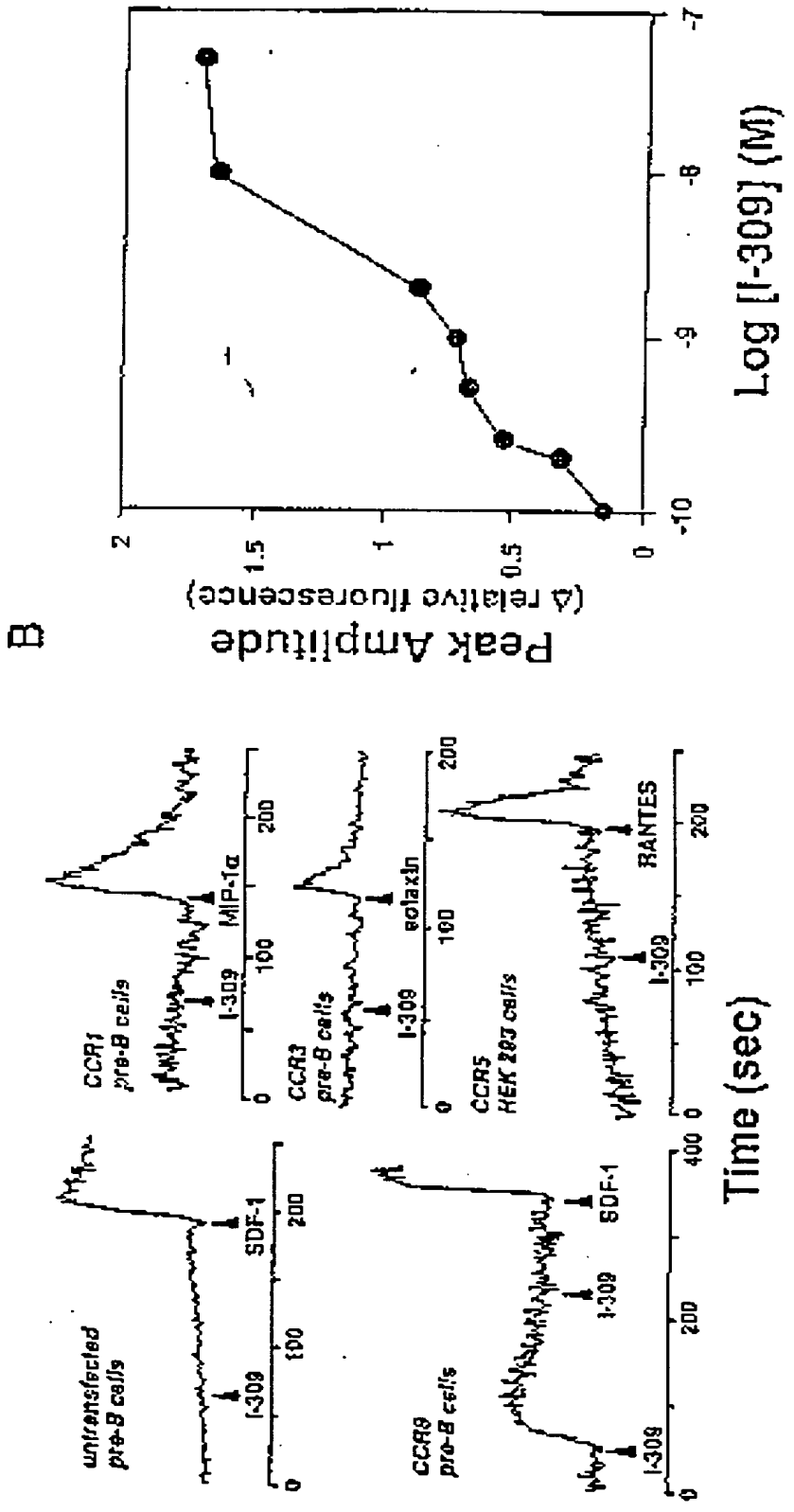


Fig .3

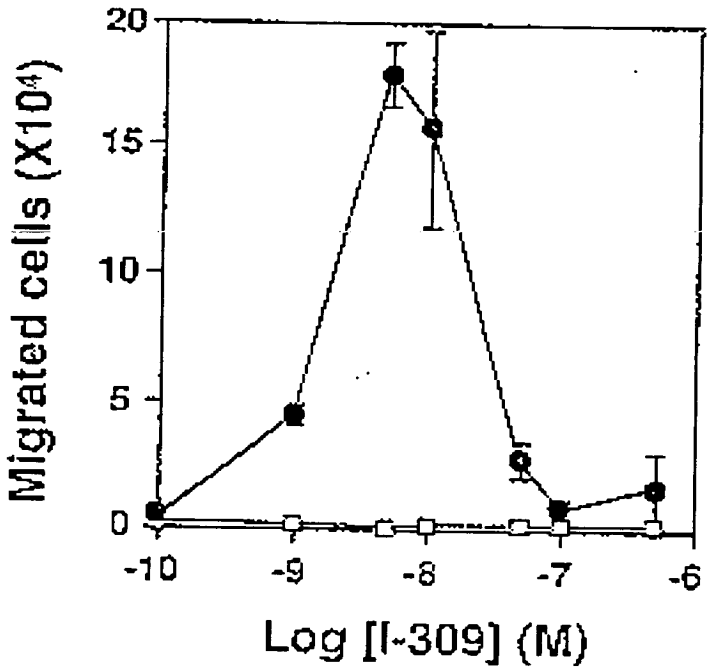


Fig.4

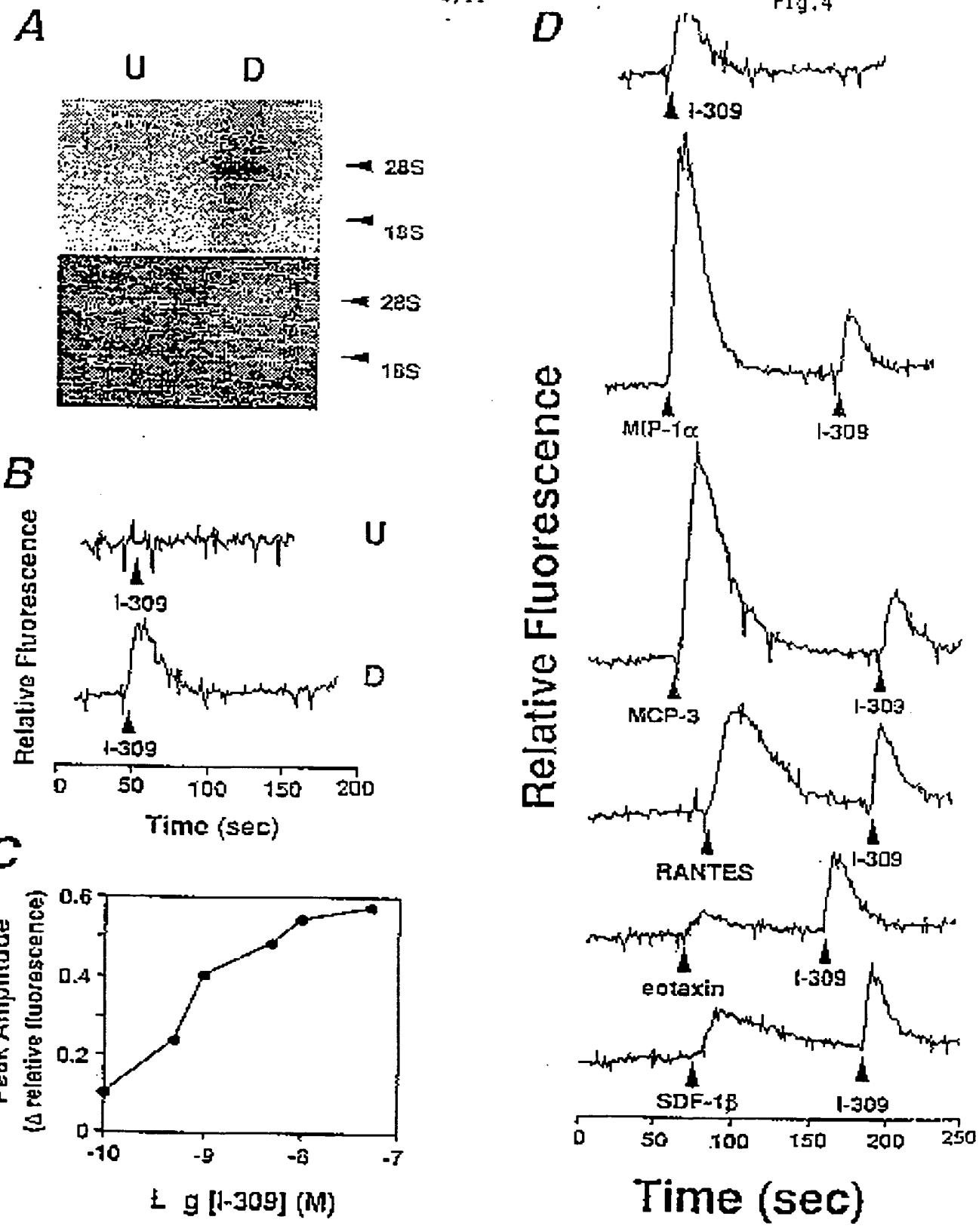


Fig.5

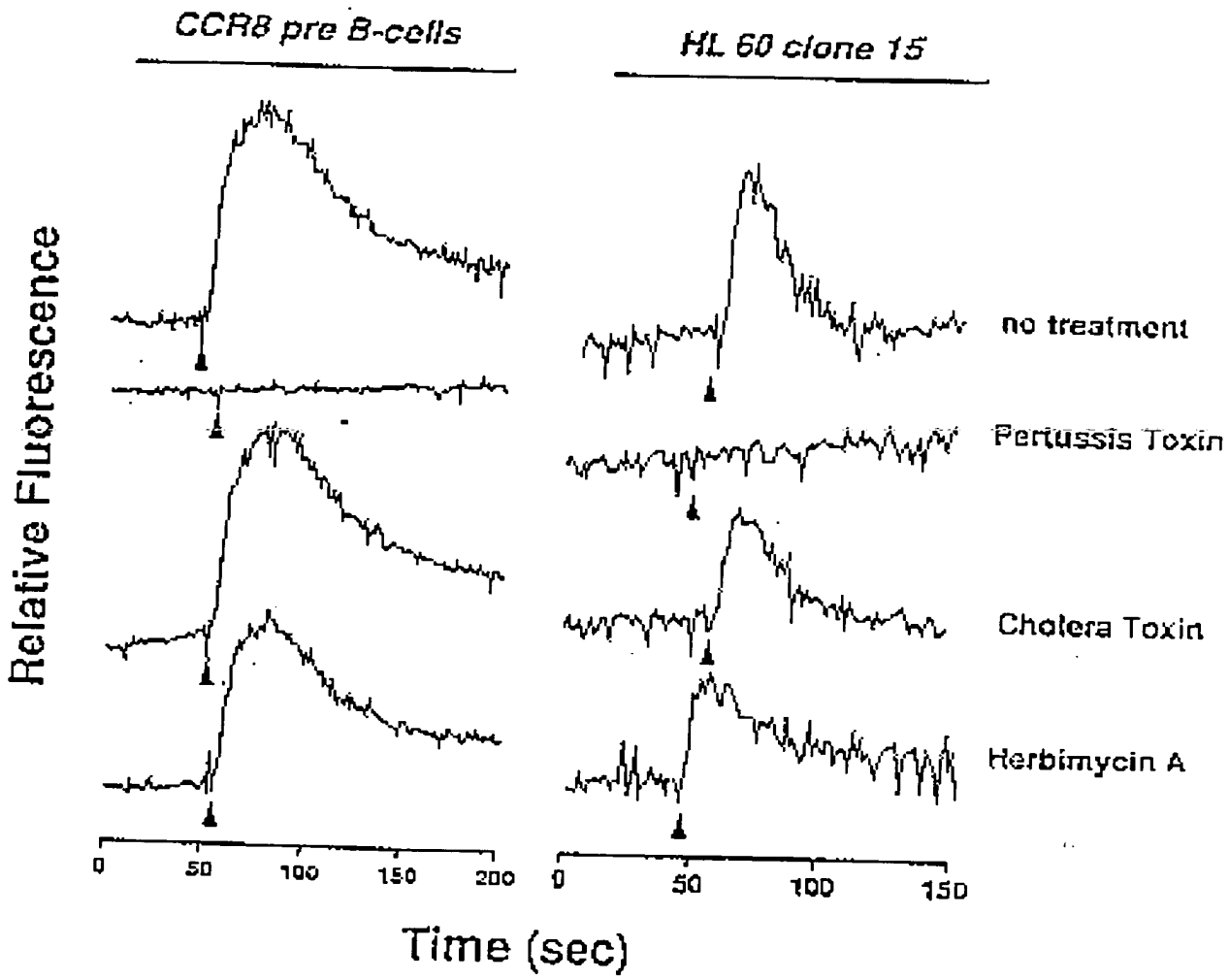


Fig.6

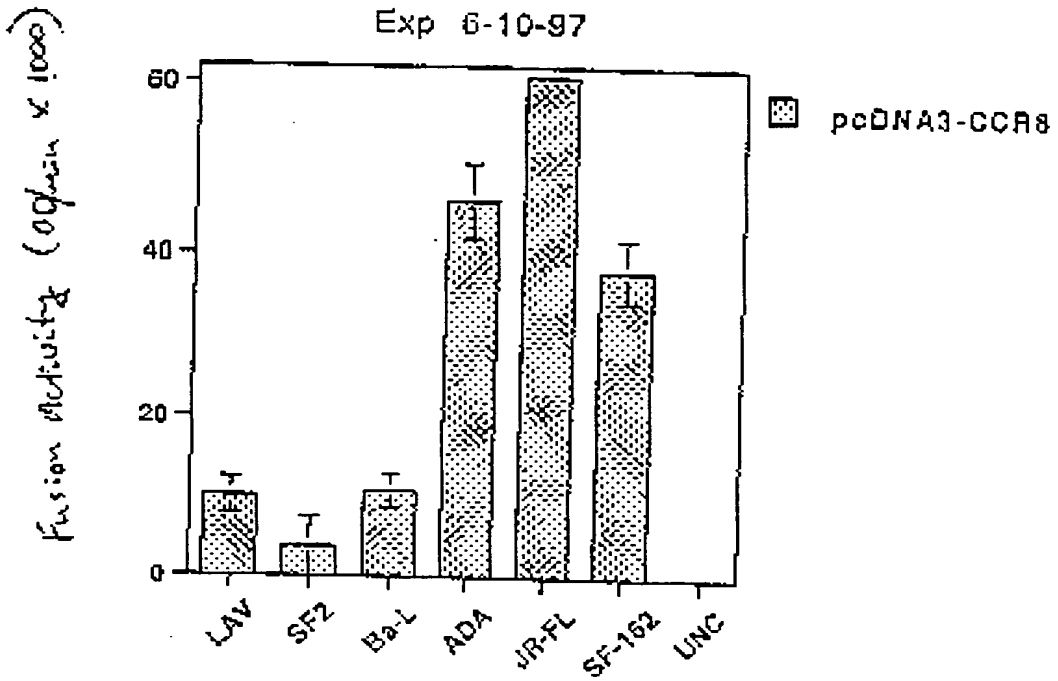


Fig.7

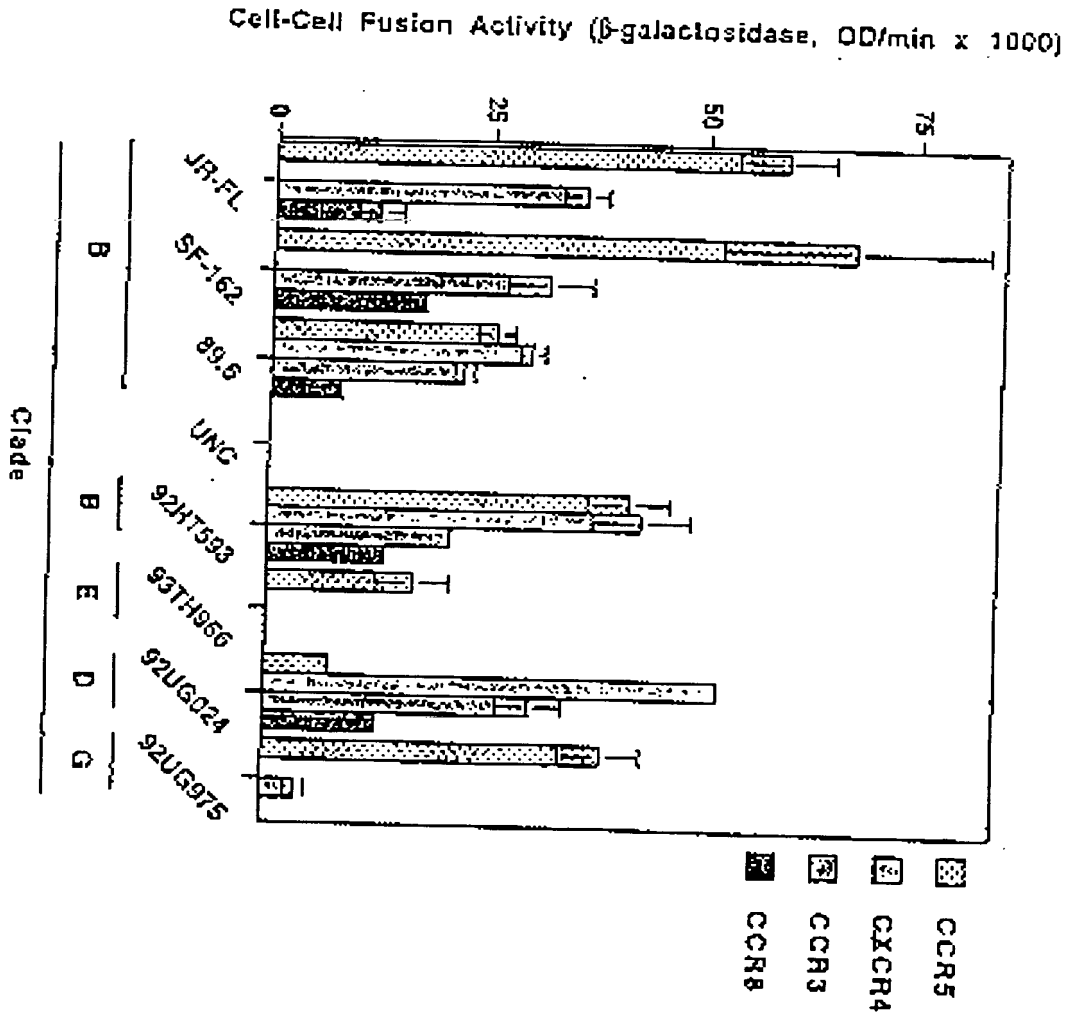


Fig.8

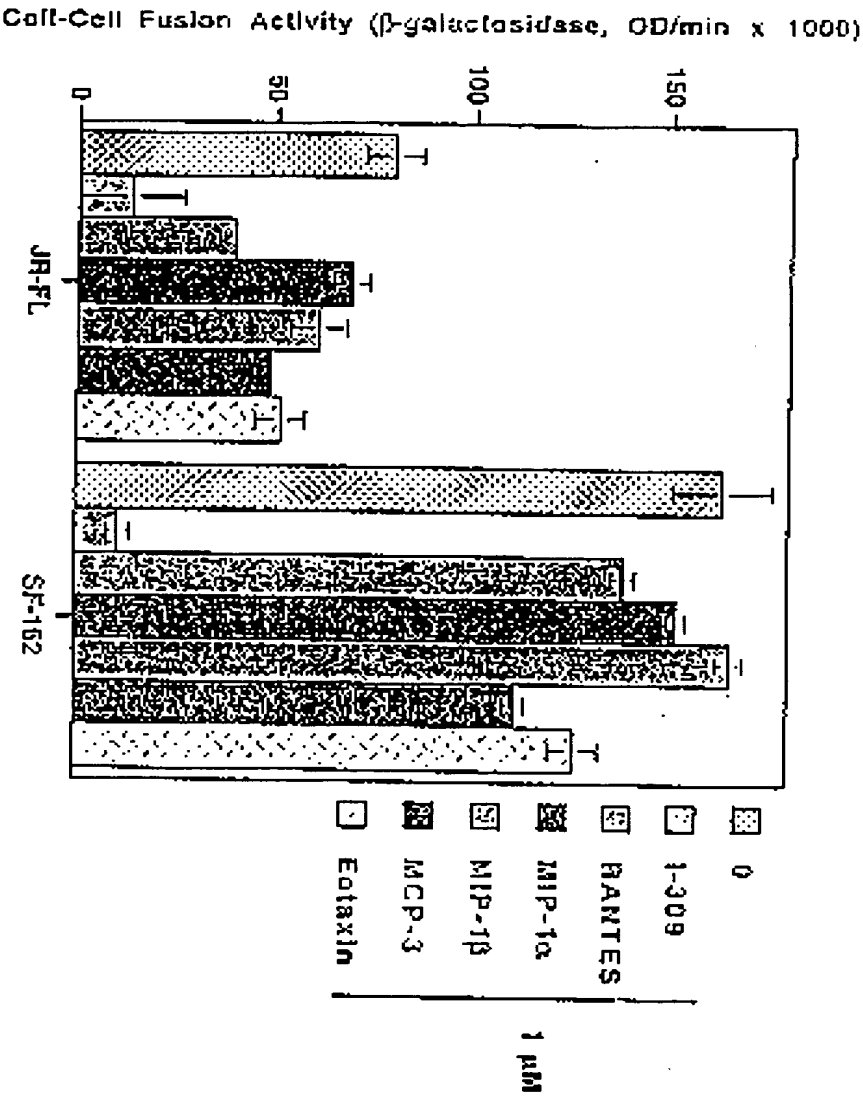
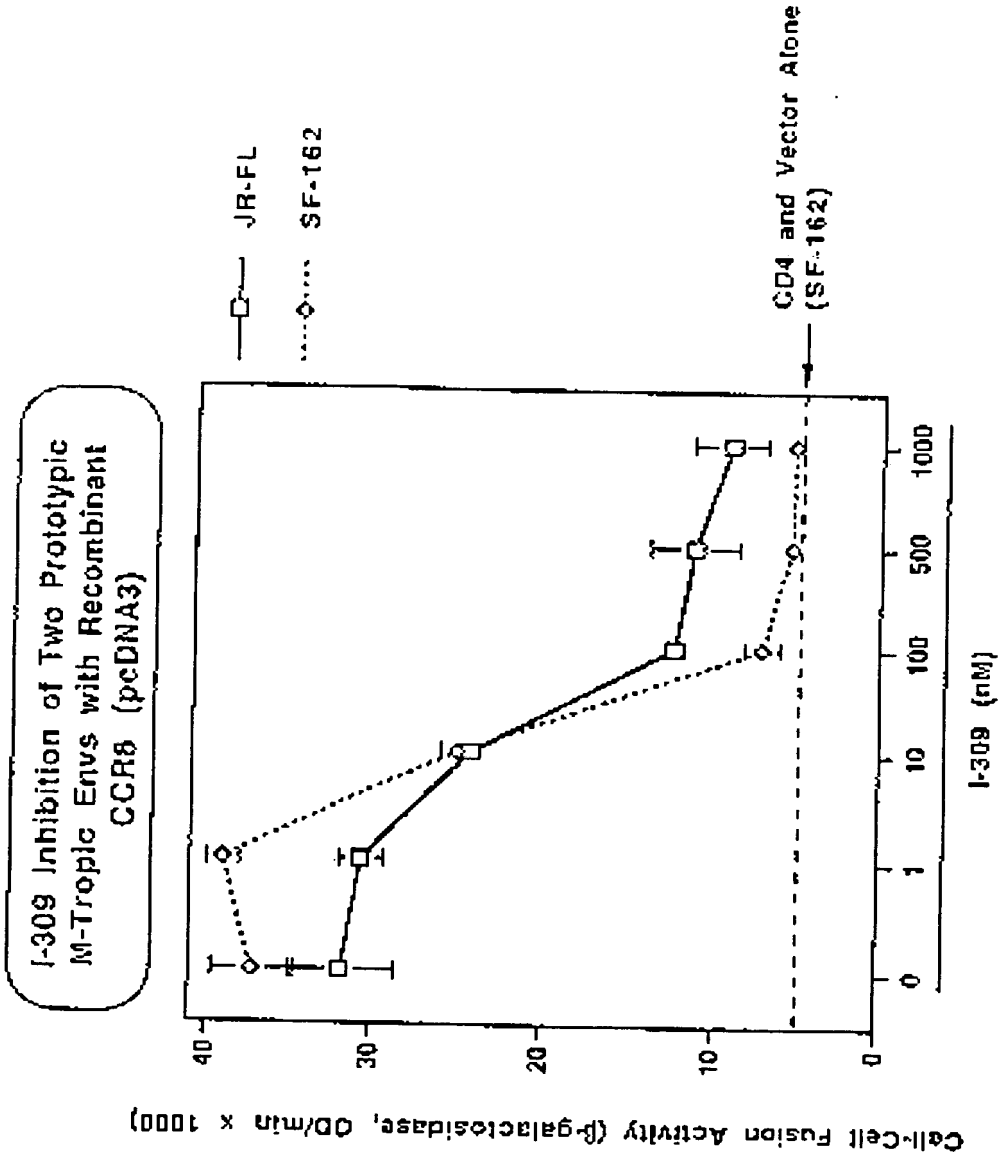

Cell-Cell Fusion Activity (β-galactosidase, OD/min x 1000)
0
50
100
150
JR-FL
SF-162
Eotaxin
MCP-3
MIP-1β
MIP-1α
RANTES
I-309
0
1 μM

Fig.9


I-309 Inhibition of Two Prototypic M-Tropic Envs with Recombinant CCR8 (pcDNA3)
JR-FL
SF-162
CD4 and Vector Alone (SF-162)
Cell-Cell Fusion Activity (β-galactosidase, OD/min x 1000)
0
10
20
30
40
0
1
10
100
500
1000
I-309 (nM)

```
   1  CCTCCCAAAG TGCTAGGATT ACAGGCATGA GCCACAGCTC CCGGTCTATC

  51  ATTTAACCTT AATTACATCT TTAAAGGCCC AAATAGTCTC ACCCACTCCA

 101  AATAGTCACA CCCACACCGG AGGTTGAGCA CTTCAACACA TGAATTTGGG

 151  GAGGACACAG TTCAGTCCAT AACATCCCCC TAATTTTTAA AAAATAAAAA

 201  TGTTTTTAAG GAGTGAATGT CTTTTATGTG TCTCTGTGAC CAGGTCCCCC

 251  TGCCTTGATG GATTATACAC TTGACCTCAG TGTGACAACA GTGACCGACT

 301  ACTACTACCC TGATATCTTC TCAAGCCCCT GTGATGCCGA ACTTATTCAG

 351  ACAAATGGCA AGTTGCTCCT TGCTGTCTTT TATTGCCTCC TGTTTGTATT

 401  CAGTCTTCTG GGAAACAGCC TGGTCATCCT GGTCCTTGTG GTCTGCAAGA

 451  AGCTGAGGAG CATCACAGAT GTATACCTCT TGAACCTGGC CCTGTCTGAC

 501  CTGCTTTTTG TCTTCTCCTT CCCCTTTCAG ACCTACTATC TGCTGCACCA

 551  GTGGGTGTTT GGGACTGTAA TGTGCAAAGT GGTGTCTGGC TTTTATTACA

 601  TTGGCTTCTA CAGCAGCATG TTTTTCATCA CCCTCATGAG TGTGGACAGG

 651  TACCTGGCTG TTGTCCATGC CGTGTATGCC CTAAAGGTGA GGACGATCAG

 701  GATGGGCACA ACGCTGTGCC TGGCAGTATG GCTAACCGCC ATTATGGCTA

 751  CCATCCCATT GCTAGTGTTT TACCAAGTGG CCTCTGAAGA TGGTGTTCTA

 801  CAGTGTTATT CATTTTACAA TCAACAGACT TTGAAGTGGA AGATCTTCAC

 851  CAACTTCAAA ATGAACATTT TAGGCTTGTT GATCCCATTC ACCATCTTTA

 901  TGTTCTGCTA CATTAAAATC CTGCACCAGC TGAAGAGGTG TCAAAACCAC

 951  AACAAGACCA AGGCCATCAG GTTGGTGCTC ATTGTGGTCA TTGCATCTTT

1001  ACTTTTCTGG GTCCCATTCA ACGTGGTTCT TTTCCTCACT TCCTTGCACA

1051  GTATGCACAT CTTGGATGGA TGTAGCATAA GCCAACAGCT GACTTATGCC

1101  ACCCATGTCA CAGAAATCAT TTCCTTTACT CACTGCTGTG TGAACCCTGT

1151  TATCTATGCT TTTGTTGGGG AGAAGTTCAA GAAACACCTC TCAGAAATAT

1201  TTCAGAAAAG TTGCAGCCAA ATCTTCAACT ACCTAGGAAG ACAAATGCCT

1251  AGGGAGAGCT GTGAAAAGTC ATCATCCTGC CAGCAGCACT CCTCCCGTTC
```

FIGURE 10A

```
1301  CTCCAGCGTA GACTACATTT TGTGAGGATC AATGAAGACT AAATATAAAA
1351  AACATTTTCT TGAATGGCAT GCTAGTAGCA GTGAGCAAAG GTGTGGGTGT
1401  GAAAGGTTTC CAAAAAAAGT TCAGCATGAA GGATGCCATA TATGTTGTTG
1451  CCAACACTTG GAACACAATG ACTAAAGACA TAGTTGTCCA TGCCTGGCAC
1501  AACATCAAGC CTGTGATTGT GTTTATTGAT GATGTTGAAC AAGTGGTAAC
1551  TTTAAAGGAT TCTGTATGCC AAGTGAAAAA AAAAGATGTC TCACCTCCTT
1601  ACATATGCAA AAATATACCT TCAGAGACTG TCAGTAGGCT GGAAGAAGTG
1651  GATATTGAAG TTTTGACATC AATGATGAGG CTCCAGTTGT CTATTCATTG
1701  ACTGATGGTG AAATGGCTGG ACTGATTCTG AATCAAGGTG ATTGTGATTA
1751  TAGTGACAAT GAAGATGATG CTATTAATAC TGCATAAAAA GTGCCTGTAG
1801  ATGACATGGT GAAAATATTT GACAGGCTTA TGGAAGGACT ACAGCAGCAC
1851  GCATTCATAA CAGAACAAGA AATTATGTCA GCTTATAAAA TCAAACAGAG
1901  ACTTCTAGAC AAAAACCATT GTTGATGAGC CAGATGCCTC TAGA
```

```
.--.          MDYTLDLSVTTVTDYYYPDIFSSPCDAELIQINGKLLLAVFYCL
LFVFSLLGNSLVILVLVVCKKLRSITDVYLLNLALSDLLFVFSFPFQTYYLLDQWVFG
TVMCKVVSGFYYIGFYSSMFFITLMSVDRYLAVVHAVYALKVRTIRMGTTLCLAVWLT
AIMATIPLLVFYQVASEDGVLQCYSFYNQQTLKWKIFTNFKMNILGLLIPFTIFMFCY
IKILHQLKRCQNHNKTKAIRLVLIVVIASLLFWVPFNVVLFLTSLHSMHILDGCSISQ
QLTYATHVTEIISFTHCCVNPVIYAFVGEKFKKHLSEIFQKSCSQIFNYLGRQMPRES
CEKSSSCQQHSSRSSSVDYIL*
```

FIGURE 10B

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, C07K 14/715, A61K 48/00, C07K 16/28, G01N 33/50, A61K 39/395, C12N 5/10

A3

(11) International Publication Number: **WO 99/06561**

(43) International Publication Date: 11 February 1999 (11.02.99)

(21) International Application Number: PCT/US98/15730

(22) International Filing Date: 29 July 1998 (29.07.98)

(30) Priority Data:
60/054,094 29 July 1997 (29.07.97) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 60/054,094 (CON)
Filed on 29 July 1997 (29.07.97)

(71) Applicant *(for all designated States except US)*: THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES, NATIONAL INSTITUTES OF HEALTH Office of Technology Transfer [US/US]; Suite 325, 6011 Executive Boulevard, Rockville, MD 20852–3804 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: TIFFANY, H., Lee [US/US]; 13910 Congress Drive, Rockville, MD 20853–2633 (US). MURPHY, Philip, M. [US/US]; 10 Hastings Circle, Rockville, MD 20850 (US). BERGER, Edward, A. [US/US]; 5820 Inman Park Circle, Rockville, MD 20852 (US). ALKHATIB, Ghalib [CA/US]; 13264 Penneagle Drive, Carmel, IN 46033 (US). BAZAN, Hernan [US/US]; Apartment 101, 3338 O Street, N.W., Washington, DC 20007 (US). BONNER, Tom, I. [US/US]; Bethesda, MD (US). LAUTENS, Laura [US/US]; The Plains, OH (US).

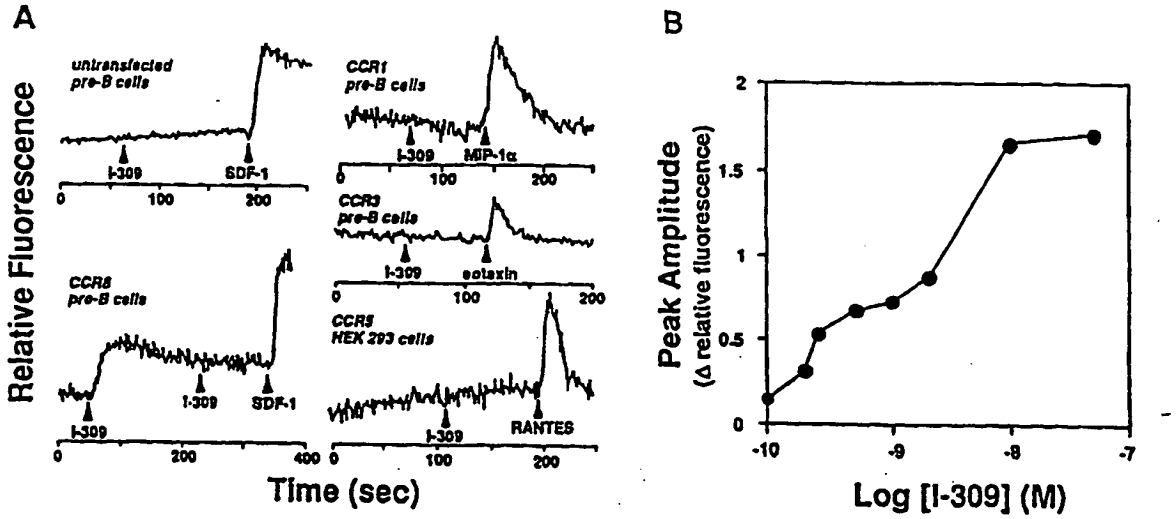
(74) Agent: HAILE, Lisa, A.; Fish & Richardson P.C., Suite 1400, 4225 Executive Square, La Jolla, CA 92037 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(88) Date of publication of the international search report:
22 April 1999 (22.04.99)

(54) Title: CHEMOKINE RECEPTOR CCR8 DNA AND USES THEREOF



(57) Abstract

The susceptibility of target to human immunodeficiency virus (HIV) infection depends on cell surface expression of the human CD4 molecule and CC chemokine receptor 8. CCR8 is a member of the 7–transmembrane segment superfamily of G–protein–coupled cell surface molecules. CCR8 plays an essential role in the membrane fusion step of infection by diverse HIV isolates. The establishment of stable, nonhuman cell lines and transgenic mammals having cells that coexpress human CD4 and CCR8 provides valuable tools for the continuing research of HIV infection. In addition, antibodies which bind to CCR8, CCR8 variants, and CCR8–binding agents, capable of blocking membrane fusion between HIV and target cells represent potential anti–HIV therapeutics.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

Internatior pplication No
PCT/US 98/15730

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/715 A61K48/00 C07K16/28 G01N33/50
A61K39/395 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TIFFANY, H. L. ET AL.: "Identification of CCR8: a human monocyte and thymus receptor for the CC chemokine I-309" JOURNAL OF EXPERIMENTAL MEDICINE, vol. 186, no. 1, 7 July 1997, pages 165-170, XP002092538 see page 166, column 1, paragraph 3 see page 169, column 1, paragraph 2 - column 2 --- -/--	1,6, 13-16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 February 1999	18/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chambonnet, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropnate, of the relevant passages	Relevant to claim No.
X	SAMSON, M. ET AL.: "Molecular cloning and chromosomal mapping of a novel human gene, ChemR1, expressed in T lymphocytes and polymorphonuclear cells and encoding a putative chemokine receptor" EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 26, 1996, pages 3021-3028, XP002092740 see specially page 3025 col. 1 paragraph 4 to col. 2 and pge 3027, paragraph 4 see the whole document ---	13,66
X	WO 96 39434 A (HUMAN GENOME SCIENCES INC ;LI YI (US); ROSEN CRAIG A (US); RUBEN S) 12 December 1996 see the whole document ---	1,5-7,9, 11, 13-16, 29,57-66
X	ZABALLOS A ET AL: "MOLECULAR CLONING AND RNA EXPRESSION OF TWO NEW HUMAN CHEMOKINE RECEPTOR-LIKE GENES" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 227, no. 3, 23 October 1996, pages 846-853, XP002055490 cited in the application see the whole document ---	1,5,29, 32,33
X	ROOS, R. STUBER ET AL.: "Identification of CCR8, the receptor for the human CC Chemokine I-309" JOURNAL OF BIOLOGICAL CHEMISTRY (MICROFILMS), vol. 272, no. 28, 11 July 1997, pages 17251-17257, XP002092741 MD US see the whole document ---	1,6, 13-16, 18-20, 32,66
P,X	HORUK, R. ET AL.: "The CC chemokine I-309 inhibits CCR8-dependent infection by diverse HIV-1 strains" JOURNAL OF BIOLOGICAL CHEMISTRY. (MICROFILMS), vol. 273, no. 1, 2 January 1998, pages 386-391, XP002092742 MD US see the whole document --- -/--	1-8,10, 11,13, 14, 16-20, 22,24, 34-37

INTERNATIONAL SEARCH REPORT

Internatior pplication No
PCT/US 98/15730

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X,P	BERNARDINI, G. ET AL.: "Identification of the CC chemokines TARC and macrophage inflammatory protein-1 beta as novel functional ligands for the CCR8 receptor" EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 28, no. 2, February 1998, pages 582-588, XP002092743 see the whole document	1,5-7, 11, 13-16, 29, 32-34, 42,48, 49,66
P,X	RUCKER, J. ET AL.: "Utilization of chemokine receptors, orphan receptors, and Herpesvirus-encoded receptors by diverse Human and Simian Immunodeficiency Viruses" JOURNAL OF VIROLOGY., vol. 71, no. 12, December 1997, pages 8999-9007, XP002092744 ICAN SOCIETY FOR MICROBIOLOGY US see the whole document	1-66
P,X	TIFFANY, H. L. ET AL.: "A model system for inducible expression of the CC chemokine receptors CCR1, CCR3 and CCR8" JOURNAL OF LEUKOCYTE BIOLOGY, no. suppl, December 1997, page 7 XP002092745 see abstract	1,6

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internat .al application No.
PCT/US 98/ 15730

Box I Observations where c rtain claims w re found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Please see Further Information sheet enclosed.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
Due to lack of characterization of the claimed compounds in the underlying application, the search had to be limited.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Although partially claims 22 to 24 , 57 to 61 and 65, as far a they concern an in vivo method, and claims 25 to 28, 51 to 56 and 63 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Info. .ion on patent family members

International Application No
PCT/US 98/15730

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9639434 A	12-12-1996	AU 2766395 A	24-12-1996
		EP 0832125 A	01-04-1998